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Chi-hsiang Lei and Hugh F. Clifford

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Contents

List of Figures, vi

List of Tables, ix

Biographical Note, xi

Résumé, xii

Summary, xiii

Acknowledgements, xiv

Part I Development of parthenogenetic eggs *in vitro* and duration of embryonic stages
Abstract, 1
Introduction, 1
Materials and Methods, 2
Results, 2
Discussion, 6
Literature Cited, 7

Part II Vital statistic properties of laboratory populations
Abstract, 13
Introduction, 14
Materials and Methods, 14
Preadult Instars, 15
Longevity, 17
Growth, 17
Reproduction, 20
Discussion, 25
Literature Cited, 27

Part III Periodicity, reproduction and growth of *D. schødleri* in the lake
Abstract, 41
Introduction, 41
Materials and Methods, 42
Description of the Study Area, 43
Zooplankton of Big Island Lake, 43
Life History of *D. schødleri*, 44
Interpretations of Length–Frequency Distributions, 47
Literature Cited, 48

List of Figures

Part I
Development of *Daphnia schødleri* eggs and embryos *in vitro*

1
Egg three hours after deposition, 8
2
Egg at six hours, 8
3
Egg at nine hours, 8
4
Egg at 12 hours, 8
5
Egg at 13 hours, 8
6
Egg at 15 hours, 9
7
Egg at 18 hours, 9
8
Embryo at 21 hours, 9
9
Embryo at 22 hours (dorsal view), 9
10
Embryo at 24 hours (dorsal view), 9
11
Embryo at 24 hours (ventral view), 10
12
Embryo at 27 hours (side view), 10
13
Embryo at 27 hours (dorsal view), 10
14
Embryo at 30 hours (dorsal view), 10
15
Side view of embryo at 30 hours, 10
16
Embryo at 33 hours (dorsal view), 11
17
Embryo at 33 hours (side view), 11
18
Embryo at 36 hours (dorsal view), 11
19
Embryo at 38 hours (side view), 11
20
Embryo at 39 hours (dorsal view), 11

21
Embryo at 42 hours (ventral view), 12
22
Side view of embryo at 43 hours, 12
23
Embryo at 57 hours, 12

Part II

1
Survival curves for male and female *D. schødleri* in instars and days, 28
2
Mean growth curves for eight male and eight female *D. schødleri*, 29
3
Absolute growth increment curves for the males and females of Figure 2, 30
4
Growth curves in days of female *D. schødleri* at 20°C and 5°C, 31
5
Growth curves in instars of female *D. schødleri* at 20°C and 5°C, 32
6
Scatter diagrams of mean carapace length and height of the males and females of Figure 2, 33
7
Coefficients of variation for each linear dimension of the *D. schødleri* of Figure 2, 34
8
Size–frequency distributions during the first seven instars of males and females reared at 25°C, 35
9
Mean number of young produced during each adult instar, 36
10
Mean brood size in relation to age of females, 37
11
Length of liberated young in different broods, 38
12
Formation of an ephippium in *D. schødleri*, 39

Part III

1
Water temperature and dissolved oxygen of Big Island Lake, 1966 and 1967, 49

2
Seasonal changes in population size of the major zooplankters of Big Island Lake, June 1966 to July 1967, 50

3
Seasonal variations in parthenogenetic egg production and body length of mature parthenogenetic females, 51

4
The relationship between size of sexual eggs and size of sexual females, and between size of parthenogenetic eggs and parthenogenetic females, 52

5
Population length-frequency histograms of *D. schødleri* in Big Island Lake, 13 June 1966 to 16 October 1967, 53

List of Tables

Part I

1
Mean and relative durations of *Daphnia schødleri*'s embryonic stages in the brood chamber, 4

2
Number of *D. schødleri* females from Big Island Lake carrying embryos, 4

3
Mean durations of brooding periods and instars for *D. schødleri* primiparous in the sixth, seventh and eighth instars, at 5°C, 5

4
Mean durations of brooding periods and instars for *D. schødleri* primiparous in fifth instar, at 20°C, 6

Part II

1
Initial size and size in first adult instar of female *Daphnia schødleri*, 16

2
Length of young in first instar and instar in which they reached maturity at 20°C, 16

3
Relative mean growth increments of total length for *D. schødleri* males and females of Figure 2, 18

4
Relationship between egg size and size of young, 20

5
Mortality of laboratory populations at 20°C and 25°C, 21

6
Female size in first adult instar and number and size of their young in first adult instar, 22

7
Reproductive features of seven females that produced at least one ephippium at 5°C, 23

8
Vital statistic properties of *D. schødleri*'s laboratory population, 24

Part III

1
Limnetic zooplankton found in Big Island Lake, other than Protozoa, opp. p.42

2
Seasonal changes in percentage composition of life cycle stages, June 1966 to July 1967, 44

3
Seasonal variations in mean sizes of parthenogenetic and gamogenetic females, 45

4
Relationship between parthenogenetic egg production and body size of mature female *D. schødleri* for different sampling dates, 46

Biographical Note

Chi-hsiang Lei

Chi-hsiang Lei, a native of Taiwan, received his B.Sc. in Fishery Biology from the National Taiwan University, Taipei, Taiwan, in 1960 and his M.Sc. in Zoology from the University of Alberta, Edmonton, in 1968. His primary research interest is in the biology and ecology of zooplankton. He is at present studying for his Ph.D at the University of Kansas, Lawrence. His current research is on the ecological energetics of *Daphnia*.

Hugh F. Clifford

Hugh F. Clifford received his B.Sc. and M.Sc. degrees from Michigan State University and his Ph.D. in 1965 from Indiana University, where he studied under Dr. David G. Frey. His special interest has been the ecology of aquatic invertebrates in seasonally dry and winterkill habitats. He is currently carrying out limnological studies on a subarctic, brown-water stream of Alberta. Dr. Clifford is at present an associate professor in the Department of Zoology at the University of Alberta.

Résumé

En combinant les relevés faits sur le terrain avec les observations de laboratoire, les auteurs ont étudié les aspects biologiques de spécimens de *Daphnia schødleri* Sars provenant d'un lac à destruction hivernale d'Alberta. À 18°C, l'ensemble de la période embryonnaire *in vitro* fut d'environ 57 heures. Trois stades de croissance caractérisaient le développement embryonnaire. Toutefois, deux stades seulement s'accompagnaient du rejet d'une membrane d'oeuf. À 5°C, la période moyenne d'incubation variait en fonction de la durée moyenne des stades adultes respectifs. Après le 16e stade, à 20°C, la période de chaque incubation est demeurée sensiblement la même, alors que celle des stades adultes ultérieurs s'est prolongée progressivement.

Les femelles élevées en laboratoire à 5°C, 20°C et 25°C présentaient de quatre à sept stades larvaires. Les mâles en avaient trois ou quatre. À 25°C, la longévité moyenne était de 36 jours (18 stades) pour les femelles, et de 41 jours (18 stades) pour les mâles. Chez les femelles, la croissance absolue la plus considérable s'est produite à la mue qui survient entre l'adolescence et le stade primipare. Chez les mâles, cette croissance s'est manifestée entre la pré-adolescence et l'adolescence. À 20°C, les femelles avaient réalisé la plus grande partie de leur croissance dès le 10e jour, alors qu'à 5°C, la courbe de croissance ne se stabilisait qu'au 40e jour. À 25°C, le nombre moyen de jeunes par ponte atteignait son maximum au quatrième stade adulte. À 20°C, il y avait deux maxima de reproduction, l'un au huitième stade adulte et l'autre au 31e. Dans le laboratoire, *D. schødleri* a pondu des oeufs fertiles à 5°C. Toutes les femelles pondant de tels oeufs traversaient un stade de stérilité immédiatement après le stade éphippial.

Dans le lac, la génération *ex ephippio* est apparue tard au printemps et, au mois de mai, on pouvait observer la parthénogénèse. On a noté deux périodes de reproduction sexuée. La plus importante a eu lieu en juin et juillet, alors que la population était maximale; l'autre, de moindre importance, s'est produite en septembre. La première période fut attribuée à la génération *ex ephippio*. Le nombre moyen d'oeufs parthénogénétiques par ponte diminua immédiatement avant le commencement de la période de reproduction sexuée. La ponte moyenne de toute la période à l'étude a été de 7,9 oeufs, ce qui est de beaucoup inférieur à la ponte moyenne des populations observées en laboratoire.

Summary

We studied the biology of *Daphnia schødleri* Sars from a winterkill lake in Alberta by combining field and laboratory data. At 18°C the total *in vitro* embryonic period was about 57 hours. During development there were three stages of embryonic size increase, but the shedding of an egg membrane could only be associated with two of these stages. At 5°C the mean duration of each brooding period varied directly with the mean duration of the respective adult instar. After the sixteenth instar at 20°C, the duration of each brooding period remained about the same, whereas the time interval of the subsequent adult instars became progressively longer.

Female *D. schødleri*, when cultured in the laboratory at 5°C, 20°C, and 25°C, had four to seven preadult instars; males had three to four preadult instars. At 25°C the average longevity was 36 days (18 instars) for females and 41 days (18 instars) for males. For females, the largest absolute growth increment was at the molt between the adolescent and primiparous instars; for males this increment was between the preadolescent and adolescent instars. At 20°C most of the growth of females was achieved by day 10, whereas the growth curves at 5°C did not level off until day 40. At 25°C, the mean number of young per brood was greatest in the fourth adult instar; at 20°C there were two peaks of young production, one in the eighth adult instar and another in the thirty-first adult instar. In the laboratory, *D. schødleri* produced sexual eggs only at 5°C. All females producing sexual eggs had a sterile instar immediately following the ephippial instar.

In the lake, the *ex ephippio* generation appeared in late spring, and by May parthenogenetic reproduction was taking place. There were two periods of sexual reproduction, a major period in June and July, at which time the population was exhibiting maximum numbers, and a minor period in September. The entire first period of sexual reproduction was accounted for by the *ex ephippio* generation. The average number of parthenogenetic eggs per brood diminished just before the onset of sexual reproduction. The average brood size for the entire study period was 7.9 eggs, which was considerably lower than the average brood size of laboratory populations.

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Part I Development of Parthenogenetic Eggs *in vitro* and Duration of Embryonic Stages

Abstract

In vitro development of *D. schødleri* Sars eggs was observed hourly throughout the embryonic period. At 18°C the total embryonic period was about 57 hours. During *in vitro* development there were three stages of embryonic size increase, but the shedding of an egg membrane could only be associated with two of these stages. Development of parthenogenetic eggs was also observed in the brood chamber of females by separating embryonic development into eight stages. Each of the eight embryonic stages at 5°C had about the same relative duration as those at 20°C. Study of various samples taken from Big Island Lake revealed that the females, at almost all times, were carrying either a larger or smaller number of embryos of a particular stage than would have been predicted from random ovulation and the relative duration of each embryonic stage at 20°C.

At 5°C the mean duration of each brooding period varied directly with the mean duration of the respective adult instar. At 20°C, where more instars were observed, the mean duration of the brooding period varied directly with the mean duration of the adult instar until the sixteenth instar; thereafter the duration of each brooding period remained about the same, whereas each of the subsequent adult instars continued to exhibit a progressively longer time interval.

Introduction

In western North America, *Daphnia schødleri* is found from Texas to the Arctic Circle. In the northern part of its range, e.g. the prairies of Alberta and Saskatchewan, *D. schødleri* is usually found in temporary ponds and small winterkill lakes, often occurring in large numbers during the ice-free season. *D. schødleri* also occurs sporadically in large northern oligotrophic lakes, e.g. Great Slave Lake; Anderson (1968) found *D. schødleri* in high elevation mountain lakes, which may possibly become depleted of oxygen in the wintertime. Very little is known about the biology of *D. schødleri* from any habitat in its northern range.

By combining field and laboratory data, we studied the life history of *D. schødleri* from a winterkill lake in central Alberta. This lake, Big Island Lake, is ice-covered for six months of the year, and winter stagnation occurs before the ice breaks up in April or May.

The study is to be reported in three parts:

I laboratory study of external embryological features and embryonic stages;

II laboratory study of growth and reproduction;

III field study of seasonal abundance, seasonal variation in egg production, and cyclic reproduction.

Part I deals with the *in vitro* development of parthenogenetic eggs, duration of embryonic stages in the brood chamber, and the duration of the brooding period.

Materials and Methods

D. schødleri used in the laboratory experiments were the third generation of a single non-ephippial female taken from Big Island Lake on 3 July 1967. In the laboratory the animals were cultured in a diluted medium of Banta's manure-soil stock (Banta 1921). Three hours after being deposited in the brood chamber, eggs were dissected out of the female under a dissecting microscope, using fine needles. The eggs were then transferred to a depression slide filled with filtered aquarium water at 18°C and observed hourly throughout the embryonic period. Several series of eggs developed *in vitro* using this method.

Duration of each embryonic stage in the brood chamber was based on 10 broods at 20°± 1°C and 46 broods at 5°± 1°C. Hereafter these temperatures are referred to as 20°C and 5°C respectively. Duration of the total brooding period at 20°C and 5°C was based on a varying number of broods, depending on the instar number (Tables 3 and 4, pp.5 and 6).

Results

In vitro development

At three hours the parthenogenetic egg of *D. schødleri* is opaque, appears yellowish green, and has a large, yellow, fat globule in its centre (Figure 1). By the six-hour stage, the egg is translucent and a transparent edge can be seen. At nine hours an invagination, which later separates the second antennae from the body proper, appears near the future anterior end of the embryo. During the next three hours, the invagination becomes more distinct; a vertical constriction appears at the extreme posterior end of the egg at 12 hours, marking the future bilaterally symmetrical plan of the embryo.

At 18 hours the abdominal appendages appear.

Body segmentation becomes more prominent at 21 hours. At this time the embryo, having just cast off the outer egg membrane, shows a slight increase in size. The second antennae are present as short, forked stubs, closely appressed to the body.

At 22 hours the prospective brain is distinguishable, and a mid-dorsal, longitudinal fold begins to thin out laterally and posteriorly to form the carapace.

At 24 hours the head bulge becomes distinguishable, and the second antennae increase in length.

A few feeble heartbeats were observed in 30-hour embryos. A grayish material appears antero-dorsad to the prospective brain at 27 hours. This material differentiates into two, very small, pink bodies (eyes) by 30 hours but because of their minute size, they are difficult to demonstrate. These pink eyes can be easily distinguished in 33-hour embryos; also at this stage, the prospective ocellus appears.

At 30 hours the caudal spine is visible, extending out from the posterior end of the prospective carapace. With subsequent development, the spine continues to increase in length and eventually will curl ventrad and anteriorly between the posterior edges of the carapace.

The joints of the second antennae first become visible in the 27-hour embryo, with

the three terminal setae of the second antennae becoming distinguishable in the 36-hour embryo.

Intestine and hepatic caeca are seen at 39 hours, although not in dorsal view. The embryo, although casting off the outer egg membrane at the 21-hour stage, remains enclosed in an inner egg membrane tending to restrict the movement of the embryo.

By the 42-hour stage, the inner egg membrane is cast off; the embryo increases greatly in body length and is capable of moving about by using the second antennae. At this time the distal portion of the second antennae separates from the body; the two rami, originally appressed to each other, also separate. The two small pink eyes gradually increase in size, becoming completely fused at 43 hours.

From 43 hours through 56 hours, no significant changes in external morphology were observed.

During the 57-hour stage, the caudal spine extends and the three terminal setae of the second antennae become longer than their rami. Although the embryo rapidly increased in size at this stage, we did not observe the casting off of another membrane. With the extension of the caudal spine, embryonic development is considered completed. At 18°C the total embryonic period is about 57 hours. All eggs of the *in vitro* study developed into females.

Embryonic stages in the brood chamber

Parthenogenetic eggs were observed being deposited into the brood chamber on 246 occasions. At 20°C, eggs were deposited into the brood chamber from two to 49 minutes after molting, the average time being 14 minutes after molting. On only seven occasions were the eggs deposited 30 minutes or more after the female had molted. When the young were released from the brood chamber, they had usually attained a development similar to that of the *in vitro* stage at 53 hours; the caudal spine was still curled and the three terminal antennal setae were still shorter than their rami.

To study embryonic development in the brood chamber, eight recognizable stages of embryonic development were selected. The stages follow those defined by Green (1956), but, because of information available from the *in vitro* study, are slightly modified and more definitive.

Stage I. Eggs opaque or translucent with transparent edges. At first eggs are gray or grayish green, but later they become yellowish green and a clear zone begins to form around the periphery (Figures 1 and 2).

Stage II. Eggs with granular transparent edges and an invagination, indicating the prospective cephalic region. Later the invagination becomes more prominent, and a constriction appears at the posterior end (Figures 3–7).

Stage III. Embryo apparent, but head not yet defined; segmentation of body prominent. In latter part of stage, antennae are present as short, forked stubs closely appressed to the body; and a thick, mid-dorsal, longitudinal fold begins to grow out forming the carapace (Figures 8–11).

Stage IV. Embryo with head bulge; antennae longer but still appressed to the body; abdominal appendages appear as stubby blocks of tissues (Figures 12–15).

Stage V. Embryo with two small pink eyes; antennae longer, still appressed to the body, but with distinct rami joints (Figures 16–17).

Stage VI. Embryo with two brown eyes; three terminal antennae setae visible; body completely enclosed in carapace (Figures 18–20).

Stage VII. Embryo with two large black eyes very close to each other; distal portion of antennae separated from body (Figure 21).

Stage VIII. Embryo with one large black eye (Figures 22 and 23), and all subsequent development while in the brood chamber.

Mean duration of each embryonic stage for animals cultured at 5°C and 20°C is shown in Table 1. Low temperatures increased the duration of each embryonic stage and hence the entire embryonic period. Each of the eight embryonic stages at 5°C had about the same relative duration as those of 20°C, the main difference being a relatively shorter Stage VIII at 5°C.

Green (1956) noted that female *Daphnia magna* Straus collected from ponds had larger numbers of embryos in particular

Table 1
Mean and relative durations of *Daphnia schødleri*'s embryonic stages in the brood chamber, based on 46 broods at 5°C and 10 broods at 20°C

	5°C		20°C	
Stage	Mean duration (hrs)	Relative duration (%)	Mean duration (hrs)	Relative duration (%)
I	64.1	16	10.1	19
II	71.0	18	8.1	15
III	45.8	11	6.1	11
IV	64.1	16	6.9	13
V	24.0	6	3.2	6
VI	42.7	11	4.7	9
VII	24.0	6	3.0	6
VIII	65.5	16	11.3	21
Total	401.2		53.4	

stages than would have been expected considering the calculated duration of each stage; he suggested that either egg laying was synchronized for the whole population or that females in the same stage of a reproductive instar tended to congregate in a particular area of the pond. For each of seven samples from Big Island Lake, taken at various times during the study, we determined the number of *D. schødleri* females carrying embryos of each of the eight embryonic stages, and then compared these actual frequencies with the expected frequencies (assuming random ovulation) of the 20°C laboratory animals, using Chi-square analysis (Table 2). Only the 26 June 1967 sample had an actual frequency that was *not* significantly different (92 per cent level) from the expected frequency. The tendency was for the actual number of one or sometimes two stages to be greatly out of proportion with the expected number, thus influencing the Chi-square analysis. This supports Green's (1956) suggestion that egg laying in *Daphnia* can be synchronous.

Brooding period

Brooding period is the length of time that developing individuals spend in the brood chamber (Anderson and Jenkins 1942). It is not necessarily the same as the embryonic period. Occasionally the developing young are released before the embryonic period has been completed. For example: in our study, although the young were usually released in a stage similar to the *in vitro* stage at 53 hours, occasionally the embryos would be released in a stage resembling the *in vitro* stage at 42 hours. These latter embryos usually survived, completing their development in the culture medium.

Low temperature increased the brooding period of *D. schødleri* (Tables 3 and 4) just

Table 2
Number of *D. schødleri* females from Big Island Lake carrying embryos of a particular embryonic stage for each of the sampling dates

	Observed Frequencies							Expected Frequency
	1966			1967				20°C
Stage	July 13	July 19	July 27	May 27	June 13	June 26	July 3	Lab Animals
I	4	14	3	18	2	9	6	19
II	4	3	10	19	10	17	31	15
III	26	6	8	9	19	5	14	11
IV	22	11	13	17	39	8	8	13
V	2	7	1	6	2	4	0	6
VI	13	9	2	7	9	4	2	9
VII	1	1	3	2	3	4	1	6
VIII	8	6	1	0	21	18	2	21
Total	80	57	41	78	105	69	64	

Table 3
Mean duration of brooding periods and instars for *Daphnia schødleri* primaparous in the sixth, seventh, and eighth instars, at 5 C

	Sixth Instar			Seventh Instar			Eighth Instar		
Instar	Mean duration of instar	Mean brooding period	Broods observed	Mean duration of instar	Mean brooding period	Broods observed	Mean duration of instar	Mean brooding period	Broods observed
	days	days	no.	days	days	no.	days	days	no.
6	13.2	12.8	5						
7	16.0	15.3	3	16.0	15.9	10			
8	15.7	15.3	3	18.7	18.2	6	17.0	16.5	2
9	19.0	18.5	2	19.4	19.2	6	18.5	18.0	2
10	18.0	18.0	1	21.0	20.0	1	17.0	17.0	1

as it increased the embryonic period. At 5°C, the mean duration of the brooding period varied with the mean duration of the adult instars, the time interval of adult instars increasing progressively with age. At 20°C, where more instars were followed, the brooding period duration progressively increased in time during the early reproductive instars, as did the duration of the adult instars. However, by the sixteenth instar the mean duration of the brooding periods had levelled off, exhibiting no further correlation with the progressively increasing time intervals of the adult instars. Since we observed that only rarely were the eggs deposited into the brood chamber 30 minutes or more after a molt, females in very late instars must have fairly long barren periods between releasing the broods and molting again, this barren period increasing as the females become older. In field studies, *Daphnia* populations are often separated into categories depending on reproductive features, one category being females of adult size but without eggs in the brood chambers; these females are usually considered to be temporarily or permanently sterile. For *D. schødleri*, the barren females are more likely to be old, slowly reproducing females instead of sterile females.

Table 4
Mean durations of brooding periods and instars for *Daphnia schødleri* primiparous in fifth instar, at 20°C

Instar number	Mean duration of instar	Mean brooding period	Broods observed
	hours	hours	no.
5	53.0	51.8	26
6	53.5	51.5	24
7	53.3	50.8	24
8	56.0	52.3	24
9	56.4	53.0	24
10	55.6	51.8	24
11	57.8	53.5	24
12	59.3	54.0	24
13	59.7	53.2	24
14	60.4	54.8	22
15	63.3	55.8	22
16	60.9	54.9	20
17	62.2	56.0	20
18	61.9	55.4	19
19	62.9	55.0	20
20	64.7	55.3	19
21	66.4	56.5	18
22	66.3	55.7	16
23	65.7	55.9	12
24	66.6	56.4	13
25	68.1	56.9	9
26	70.9	56.3	12
27	70.9	56.2	13
28	69.6	54.8	13
29	73.2	55.2	14
30	67.9	54.5	9
31	69.6	55.5	11
32	68.1	54.9	6
33	68.6	53.9	6
34	68.2	52.1	3
35	69.4	52.4	2
36	72.8	54.5	6
37	71.3	54.8	5
38	79.6	56.6	5
39	77.7	55.3	4
40	125.7	55.1	2

Discussion

The origin and significance of *Daphnia's* egg (or embryonic) membranes are not well understood. Shiino (1968) states that cladocerans have one egg membrane, a vitelline membrane. Lebedinsky (1891) describes a second membrane for *D. similis* Claus, the second membrane being called a chorion. Obreshkove and Fraser (1940), studying the *in vitro* development of *D. magna* parthenogenetic eggs, report two egg membranes, a thin, inner, vitelline membrane and a thicker, outer, egg membrane (equivalent to Lebedinsky's chorion); these workers do not mention the casting off of either membrane. Several other workers have observed both inner and outer membranes (sometimes called larval and ovular membranes respectively) surrounding cladoceran eggs without describing the shedding of either membrane. During *in vitro* development of *D. magna* eggs, Davis (1968) observed the process of size increase and consecutive shedding of two membranes. Esslova (1959) studied the *in vitro* embryonic development of *D. pulex* (de Geer) parthenogenetic eggs and found three membranes called the egg membrane (outer) and first and second (inner) embryonic membranes. All three membranes were observed being cast off, the second embryonic membrane being cast off during late embryonic development when the caudal spine extends and the embryo increases rapidly in size. Esslova equated the casting off of all three membranes to three prenatal molts.

During the development of *D. schødleri* eggs, we observed two membranes, both of which were cast off (the cast-off outer membrane is seen in Figure 8); the embryo subsequently increased in size after each cast. Although the exact nature of these membranes is not known, we consider the casting off of the membranes to be part of the hatching process and not true prenatal molts. *D. schødleri* embryos also increased rapidly in size after extension of the caudal spine, but we did not observe the casting off of a membrane at this time.

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Development of *Daphnia schødleri* eggs and embryos *in vitro*
All pictures were taken under a compound microscope at a magnification of 100

Figure 1
Egg three hours after deposition.

Figure 2
Egg at six hours, with a transparent periphery.

Figure 3
Egg at nine hours, showing at upper-right surface the first invagination.

Figure 4
Egg at 12 hours; the first demarcation of the future cephalic region. A constriction (a transparent spot) at the extreme posterior end marks the beginning of the bilaterally symmetrical plane of development.

Figure 5
Egg at 13 hours.

Figure 6
Egg at 15 hours, showing a clear constriction at the posterior end and further demarcation of the cephalic portion.

Figure 7
Egg at 18 hours; further demarcation of the cephalic and abdominal appendages.

Figure 8
Embryo at 21 hours; further demarcation of the cephalic and abdominal appendages and a more prominent bilaterally symmetrical development. The embryo has just cast off the outer egg membrane but it is still enclosed in the inner egg membrane. The cast-off egg membrane is at the side of the embryo. A blastodermic thickening begins to appear at the cephalic region, giving the first external evidence of brain development.

Figure 9
Embryo at 22 hours (dorsal view); further demarcation of abdominal appendages and development of prospective brain. The antennae appear as short, forked stubs, closely appressed to the body. The thick, mid-dorsal, longitudinal fold has begun to thin out laterally and posteriorly to form the carapace.

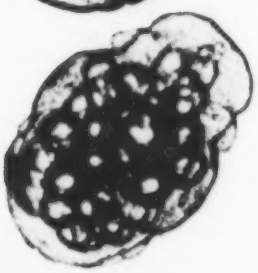

Figure 10
Embryo at 24 hours (dorsal view); further development of brain mass, carapace and other structures.

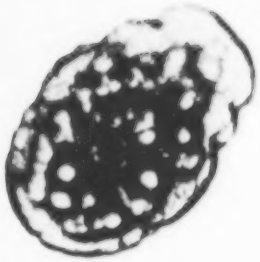

Figure 11
Embryo at 24 hours (ventral view), showing the formation of labrum and mandibles. The labrum appears as a round lobe in the cephalic region; the two mandibles appear as two smaller lobes, one on each side of prospective labrum.

Figure 12
Embryo at 27 hours (side view), showing the abdominal appendages appearing as stubby blocks of tissue. Brain mass can be seen clearly in the cephalic region. A grayish mass of granular substance appears anterodorsally to the prospective brain mass.

Figure 13
Embryo at 27 hours (dorsal view), showing a definite head bulge, and further development differentiations.

Figure 14
Embryo at 30 hours (dorsal view); further development of carapace; the caudal spine has now appeared at the posterior end of carapace. Joints of antennal rami are also distinguishable but not in focus.

Figure 15
Side view of embryo at 30 hours (ventral side up).

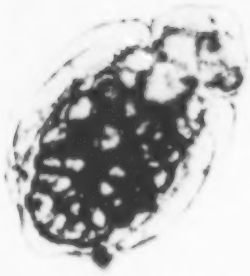

Figure 16
Embryo at 33 hours (dorsal view): the appearance of the two small pink eyes, and, posteriorly, the ocellus (the black body in front of the brain mass).

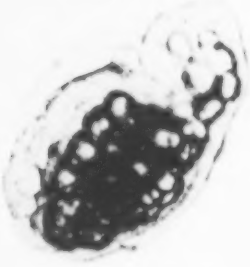

Figure 17
Embryo at 33 hours (side view, ventral side up). The ocellus appears as a small pigment body ventral to the brain mass, and two eyes are located just antero-dorsal to the brain mass, although difficult to locate in this picture.

Figure 18
Embryo at 36 hours (dorsal view), showing two distinct brown eyes.

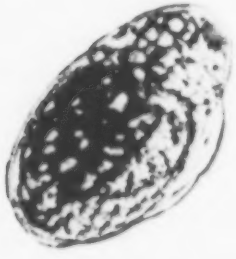

Figure 19
Embryo at 38 hours (side view), showing an infolding of the cephalothorax. The carapace now covers nearly all the body. The caudal spine is curled ventrad and anteriorly.

Figure 20
Embryo at 39 hours (dorsal view); further differentiation of the two eyes. Embryo is still enclosed in the inner egg membrane.

Figure 21
Embryo at 42 hours (ventral view), after casting the inner egg membrane. The two eyes are nearly fused. Distal portion of antennae have completely separated from the body.

Figure 22
Side view of embryo at 43 hours, showing the fused compound eye. The curled caudal spine adheres closely to the postabdomen. The three, terminal, antennal setae are still shorter than their rami.

Figure 23
Embryo at 57 hours, representing a fully developed young. The caudal spine has extended, and the three terminal, antennal setae have become longer than the terminal ramus.

Abstract

The female offspring of *Daphnia schødleri* Sars when cultured in the laboratory at 5°C, 20°C, and 25°C, had four to seven preadult instars; males had three to four preadult instars. At 25°C the average longevity was 36 days (18 instars) for females, and 41 days (18 instars) for males; at 20°C the average longevity for females was 52 days (21 instars).

As the initial size (total length in first instar) of neonate females increased, they tended to become mature in earlier instars. For females, the largest absolute growth increment was at the molt between the adolescent and primiparous instars; for males this increment was between the preadolescent and adolescent instars. The greatest relative growth increment for females was prior to the preadolescent instar; for males the increment was between the preadolescent and adolescent instars.

At 20°C most of the growth of females was achieved by day 10, whereas the growth curves for 5°C females did not level off until day 40. The coefficients of variation for carapace length, height, and total length were highest in the preadult instars for both males and females; there was no evidence that carapace length or height was a less variable measurement than total length. Fitting data of these linear dimensions to the allometric equation indicated that both male and female carapace length and female height increased in size relatively more rapidly than total length; the relationship between male height and total length was isometric. The first four instars of both males and females were easily determined from size–frequency distributions (when size–class limits were narrow), but the association between instars and size modes was not so clear-cut in later instars.

At 25°C, the mean number of young per brood was greatest in the fourth adult instar; at 20°C there were two peaks of young production, one in the eighth adult instar and another in the thirty-first adult instar. For a 110 day unit of time, mean total young production was 48 at 5°C, 378 at 20°C, and 234 at 25°C. Larger females produced more and larger young during the first adult instar than smaller females. There was a tendency for the size of the young to increase with increasing age (in instars) of females when the population was followed through 12 instars at 20°C; but for the one female followed for 34 instars, there was a sharp decline in average size of young after the twenty-third instar.

In the laboratory *D. schødleri* produced sexual eggs only at 5°C. All females producing sexual eggs had a sterile instar immediately following the ephippial instar.

Introduction

Part II of the study of *Daphnia schødleri* Sars, deals with the laboratory study of various life history phenomena (vital statistics) of growth, reproduction and related features. We were interested in comparing vital statistic properties of females with those of males, and also *D. schødleri's* properties with those of other species. In addition, we felt that certain aspects of the laboratory study might be valuable for interpreting aspects of *D. schødleri*'s biology in the lake. This last-mentioned aspect is covered in Part III. Part I covered the *in vitro* development of parthenogenetic eggs, duration of embryonic stages in the brood chamber, and the duration of the brooding period.

Materials and Methods

Laboratory experiments were carried out under three temperature conditions: 5°±1°C, 20°±1°C, and temperatures fluctuating between 22°C and 29°C with a mean temperature of 25°C; the three temperature conditions are hereafter referred to as 5°C, 20°C and 25°C. The male and female *D. schødleri* used in the culture experiments at 25°C were descendants of a single parthenogenetic female collected from Big Island Lake on 22 June 1966. This field female, shortly after being brought into the laboratory, released five female neonates; the subsequent offspring (the second generation of the field female) of these five females were used in the laboratory experiments at 25°C. After releasing the five female neonates, the field female released 31 eggs into the brood chamber; these eggs were dissected out and allowed to develop in filtered aquarium water. All of the 24 eggs that hatched developed into males. These were the males used in the laboratory experiments at 25°C. *D. schødleri* females used in the experiments at 20°C and 5°C were the third generation of a parthenogenetic female taken from Big Island Lake on 3 July 1967.

The young daphnids were isolated a few hours after being released from the brood chambers and cultured individually in 25 ml of the diluted medium of Banta's manure-soil stock (Banta 1921). On alternative days, the total volume of the medium was changed.

Measurements were made at the time of isolation and daily thereafter by placing the individuals in a depression slide with a drop of culture medium. One drop of saturated chloretone solution was added to impede activity of the 25°C animals; this was not needed at 20°C and 5°C. Measurements were made with a calibrated ocular micrometer. Total length was the distance from the apex of the head to the base of the spine; carapace length was the greatest length of the carapace exclusive of the spine; height was the shortest distance between the two lines tangential to the carapace.

D. schødleri cultured at 25°C and 5°C were examined and appropriate observations made at least once a day. The animals kept at

20°C were observed hourly from 0800 to midnight each day from the time the animals passed as eggs into their mother's brood chamber to the end of their adult life.

Preadult Instars

The number of preadult instars for female *D. schødleri* varied from four to seven. There was a tendency for females to become primiparous in earlier instars at higher temperatures (Table 1). When first primiparous, females ranged in size from 1.44 to 2.41 mm; those becoming primiparous in early instars (e.g. fifth) were generally smaller than females becoming sexually mature in later instars (e.g. eighth). For the same temperature and primiparous instar, the seventh, the mean size of ephippial females was smaller than that of parthenogenetic females.

At 20°C (the temperature at which the majority of animals were cultured) there was a relationship between size in the first instar and the instar number in which sexual maturity was reached (Table 2). The larger the female in the first instar, the fewer the number of preadult molts. Also, for a given primiparous instar, there was a positive correlation between size in the first instar and size in the first adult instar. For example, for the 57 females primiparous in the fifth instar at 20°C, the correlation coefficient was 0.7807 and is significant at the 99 per cent level.

Males were followed from the first instar to sexual maturity only at 25°C, 21 males becoming sexually mature in the fourth instar and two in the fifth instar. Sexual maturity in males was determined by the presence of sperm in the testes, the shape of the ventral margin of the carapace, and the size of the hook on the first thoracic leg.

Table 1
Initial size, and size in first adult instar of female *Daphnia schødleri*

Temperature	Number of animals	First clutch of eggs laid	Mean length in first instar	Mean length in first adult instar
		instar	mm	mm
25°C	22	5	0.548	1.640
	8	6	0.544	1.739
20°C	57	5	0.640	1.798
	27	6	0.587	1.805
	1	7	0.488	1.755
5°C	6	6	0.700	2.075
	10	7	0.619	2.199
	3	7*	0.644	1.911
	2	8	0.634	2.308

*Produced ephippia

Table 2
Length of young in first instar and instar in which they reached maturity, at 20°C

Length in first instar (mm)	Number reared	Number mature in 5th instar		6th instar		7th instar	
		no.	%	no.	%	no.	%
0.455–0.488	1			1	100.0		
0.488–0.520	3			2	66.7	1	33.3
0.520–0.553	7	2	28.6	5	71.4		
0.553–0.585	12	8	66.7	4	33.3		
0.585–0.618	8	5	62.5	3	37.5		
0.618–0.650	30	22	73.3	8	26.7		
0.650–0.683	9	6	66.7	3	33.3		
0.683–0.715	8	7	87.5	1	12.5		
0.715–0.748	2	2	100.0				
0.748–0.780	1	1	100.0				
0.780–0.813	4	4	100.0				

Longevity

At 25°C, males and females had approximately the same life-span and went through almost the same number of instars. (Fig. 1). In respect to instars, mortality of females was fairly constant throughout the life-span; for males, mortality was greatest between the fifteenth and twentieth instars.

Forty-six females were also followed at 20°C. When compared to the population at 25°C, females at 20°C lived much longer and went through many more instars, the mean life-span in days and instars being 52 and 21 respectively, with maximum longevity of 113 days and 41 instars. In addition, one female at 5°C survived for 207 days.

The 41 instars (for two females) of *D. schødleri* at 20°C possibly represent the highest number of instars recorded for a species of *Daphnia*. Possibly the large number of instars observed in the laboratory was due to a lack of crowding in our cultures, each animal having been cultured individually in a small dish.

Growth

Growth of females and males at 25°C

Figure 2 shows the mean growth curves of total length, carapace length, and height for eight females primiparous in the sixth instar at 25°C. Growth curves for the three morphometric dimensions were of the same shape. *D. schødleri* females in the experiment had an average total length of 0.52 mm immediately after being released from the brood chambers; for the remainder of the life-span, females increased 2.41 mm in mean total length, of which 1.57 mm, or 65 per cent was attained by the eighth instar.

For females, absolute growth increments for total length increased through the fifth instar, then gradually decreased until the fifteenth instar, and fluctuated for the remainder of the life-span (Fig. 3). The maximum absolute growth increment therefore was at the molt between the adolescent (fifth) and primiparous (sixth) instars for *D. schødleri* females. However, relative growth increments of total length were greatest in earlier instars, between instar two and three and between instar three and four (Table 3); relative growth increments then decreased rapidly for the remainder of the females' life-span.

Anderson, Lumer and Zupancic (1937), by calculating coefficients of correlation, determined possible relationships for *D. pulex* females between (a) initial size (i.e. total length in first instar), (A) final size (total length in twentieth instar), (t) duration of growth (the number of instars required to attain a length of 0.8A), and (i) initial velocity of growth (increment between first and second instars). They found possible inverse relationships between initial size and initial velocity of growth, and between duration of growth and final size. We performed similar calculations using data for the eight female *D. schødleri* that lived for at least 20 instars at 25°C, and obtained the following coefficients of correlation:

$r_{aA} = 0.1792$, $r_{ai} = 0.5965$, $r_{Ai} = 0.3338$, $r_{at} = -0.5157$, and $r_{At} = 0.6601$.

Table 3
Relative mean growth increments of total length for *D. schødleri* males and females of Figure 2

	Mean relative increment (%)		Number of animals	
Instars	females	males	females	males
1–2	26.9	11.8	8	8
2–3	28.8	24.7	8	8
3–4	30.6	15.1	8	8
4–5	25.9	8.9	8	8
5–6	20.7	5.2	8	8
6–7	10.1	3.5	8	8
7–8	11.8	2.8	8	8
8–9	6.2	2.7	8	8
9–10	5.4	3.2	8	8
10–11	3.0	3.2	8	8
11–12	2.9	2.5	8	8
12–13	2.4	1.8	8	8
13–14	2.4	1.8	8	8
14–15	2.3	2.3	8	8
15–16	1.1	1.1	8	8
16–17	1.1	1.7	8	8
17–18	1.8	1.1	8	8
18–19	1.8	1.6	8	7
19–20	0.7	1.6	8	6
20–21	0.1	1.0	6	4
21–22	1.4	1.6	6	4
22–23	0.2	1.5	5	4
23–24	–0.1	–1.5	5	2
24–25	0.3	1.5	4	2
25–26	0.1	1.5	4	1
26–27	1.6		3	

None was significantly different from zero (t test, 95 per cent level), indicating no evident relationships among these characteristics in *D. schødleri* females.

The mean growth curves for the three morphometric dimensions of males were all of the same shape (Fig. 2). The male growth curves were less sigmoid in shape than were the growth curves of females. The male *D. schødleri* had a mean total length of 0.76 mm in the first instar; by the eighth instar they had a mean total length of 1.49 mm, which was 73 per cent of their final mean total length. The inflection point of each curve came earlier for males than for females. It was found, in measuring total length, that both the maximum absolute growth increment and the maximum relative growth increment came between the preadolescent (second) and the adolescent (third) instars (Fig. 3 and Table 3).

The degree of interdependency, if any, between initial size, final size, duration of growth, and initial velocity of growth was also tested for males, using data for the six males that lived for 20 or more instars at 25°C. The following coefficients of correlation were obtained:

$r_{aA} = 0.5787$, $r_{ai} = -0.2697$, $r_{Ai} = -0.0623$, $r_{at} = -0.2501$, and $r_{At} = 0.5237$.

The results were the same as those described above for the females—none of the male coefficients of correlation was significantly different from zero.

Growth of females primiparous in different instars at 20°C and 5°C

High temperature increased the growth rate of *D. schødleri* by shortening the duration of each instar, growth per day being much more rapid at 20°C than at 5°C (Fig. 4). At 20°C most of the growth was achieved by day 10, whereas the growth curves for females at 5°C did not level off until about day 40. At the same temperature, 5°C, the growth curves for females becoming primiparous in different instars were similar, except for females becoming primiparous in the seventh instars and producing ephippial eggs instead of parthenogenetic eggs. For these ephippial females, growth was slower, especially from the adolescent instar (instar six at day 36) on.

Although there was much more growth per day at 20°C, growth at the end of the eleventh instar was slightly greater at 5°C (Fig. 5). Observations at 5°C were terminated when the females were in the eleventh and twelveth instars; hence their final size is not known. By extrapolating from the growth curves of Figure 4, it is estimated that the 5°C females, with the exception of ephippial females, would have to live for approximately 170 days to attain a final size equal to the final size of the 20°C animals. Such a life-span for *D. schødleri* at 5°C is certainly possible (See "longevity", p.17), at least for laboratory populations. The slower growth of ephippial females from the adolescent instar

on, both per day and per instar, would seem to support Berg's depression hypothesis (1934); i.e. ephippial females are in a state of depression.

Relationships and variability of linear dimensions

Total length is the most common linear measurement used to determine size–frequency distributions of Daphnia; total length is also usually used for most of the other ecological and physiological work of Daphnia, where an index of body size is needed. Because body shape will vary between species and may vary within the same species (obviously so for those species exhibiting cyclomorphosis), total length may not in all cases be the best index of size increase for Daphnia. Carapace length and height (and body weight) can also be used as indices of Daphnia's body size. Figure 2, p.29 gives no indication that the growth of the carapace and increase in height for both male and female *D. schødleri* are different from increase in total length.

The relationships between carapace length and total length and between height and total length were nearly linear when data of Figure 2 were plotted logarithmically (Fig. 6). Using the least-square method, data for these relationships were fitted to the power, or allometry, equation, using the log form

$$\log_e Y = \log_e b + k \log_e X$$

where b is the value of Y (carapace length or height), when X (total length) equals unity, and k is the ratio of the specific growth rates of Y and X. The values of $\log_e b$ and k (and k's 95 per cent confidence intervals) are shown in Figure 6. The relation between male height and total length is isometric ($k = 1$); the other relationships are nearly but not exactly isometric, both male and female carapace length and female height increasing in relative size slightly more rapidly than total length. The above constants are based on the assumption that simple allometry, for the linear dimensions tested, holds throughout the life-span of *D. schødleri*. There is some indication from Figure 6 that there are deviations from simple allometry, especially during the early instars. However this is not treated further in the report.

We also examined the relative variability of the three linear dimensions for the eight female and eight male *D. schødleri* of Figure 2, p.29, calculating the linear dimensions' coefficients of variation (CV) for each instar (Fig. 7). There was no evidence that either carapace length or height was a less variable measurement than total length; in fact for the preadult instars, especially males, total length was the least variable of the three measurements. CV values of all measurements were highest in the preadult instars for both males and females, and it was in the preadult instars that both the maximum absolute and maximum relative growth increments occurred.

Are any of these relatively high CVs of the early instars statistically significant? Lewontin (1966) showed that the variance of the logarithms (natural or common) of measurement gives a measure of relative variability that can be used for statistical tests; he also pointed out that for CVs of 30 or less, the square of the CV closely approximates the variance of natural logarithms. Squaring each CV value of each linear dimension used to construct Figure 7, we tested the following hypothesis for each sex of *D. schødleri*: within a particular instar none of the linear dimension's squared CVs is significantly larger (one-sided "F" test, 95 per cent level) than the smallest squared CV of that stage. Significant variability was found only in the first two instars of males, i.e. height (vs. total length) in the first instar, and height (vs. total length) and carapace length (vs. total length) in the second instar. Therefore, of the three linear dimensions, total length would be the most satisfactory measurement for documenting changes in *D. schødleri*'s body size.

Size–frequency distributions

In field studies of Cladocera life histories, information is often obtained by analyzing size–frequency distributions, i.e. plotting the number of individuals against size, usually total length, with the resulting graph exhibiting a number of size modes, which are taken as growth stages or instars. We compared the

results obtained by size–frequency distributions, where discrete instars are not known, with size–frequency distributions of individuals of discrete instars. For each of the first seven instars, total lengths of 32 females reared at 25°C were measured to the nearest 0.003 mm (0.1 micrometer unit) and the females were grouped into 0.06 mm (two micrometer units) size classes by instar, resulting in a size–frequency distribution by discrete instars. Then the 32 total-length values for each of the seven instars were lumped together. This resulted in a "composite" size–frequency distribution, which would be similar to that obtained from field samples where discrete instars are not known. The same procedure was used for 24 males reared at 25°C. Results are shown in Figure 8.

The modes of the composite distribution did not correspond exactly to the mean total length of the instars. However, for the first four instars of both females and males, each instar is easily recognized from a corresponding distinct mode in the composite distribution; in later instars this relationship is not so clear-cut, male instars five through seven being almost completely indistinguishable in the composite distribution. Overlapping of the values of the discrete instars tends to increase with instar number. If the size–class limits were to be expanded, e.g. from 0.06 mm to 0.10 or 0.25 mm, overlapping would be accentuated and the composite curve would be further smoothed; consequently it would be very difficult, if not impossible, to associate distinct instars with modes of the composite curve. And, of course, this would be the case if the sexes were not separated, assuming males made up a substantial part of the field population. In short, for *D. schødleri*, size–frequency distributions are most indicative of early instars when the size–class limits are rather narrow.

Reproduction

Natality throughout the life-span of *D. schødleri*

Natality of *D. schødleri* was based on the number of young released from the brood chamber during each instar. The number of young released may be smaller than the actual number of eggs produced per instar, since occasionally non-viable eggs were found, especially during the later instars. But it was impossible to count accurately the number of eggs in the brood chambers of living *D. schødleri*; and since non-viable eggs should not be included in the count it was felt that term young production, instead of egg production, was more appropriate for this section. Also it was established that the size of the young, when released from the brood chamber, was proportional to the size of the eggs. This was done by dissecting out the eggs from 10 females, determining individual egg volumes, and allowing the eggs to develop in filtered aquarium water; the total length of each of the newly hatched young was then measured, and the results, given in Table 4, indicate that the size of the young is correlated with the size of the egg. The calculated coefficient of correlation was 0.970 and is significant at the 99 per cent level.

Generally, the mean number of young produced per brood at 25°C increased progressively during the early adult instars

Table 4
Relationship between egg size and size of young for each of 10 *D. schødleri* females

Number of eggs measured	Number of young measured	Mean egg volume ($mm^3 x 10^3$)	Mean length (mm) of newly hatched young
13	13	6.175	0.639
12	11	5.540	0.631
9	5	5.423	0.599
12	12	7.672	0.683
7	5	5.691	0.618
10	9	8.440	0.703
10	9	6.006	0.615
6	6	11.012	0.771
14	13	7.440	0.677
10	6	5.138	0.563

and then gradually decreased in the later instars (Fig. 9). The mean number of young per brood was greatest in the fourth adult instar; the mean number per brood then fluctuated for the following 10 instars and thereafter rapidly decreased. The rapid decrease in mean number of young per brood coincided with the initial and thereafter continuing mortality of the laboratory population (Table 5). The sequence was different for the population that was followed at 20°C, and which lived for many more instars. In that case there were two peaks of young production, one in the eighth adult instar and another in the thirty-first adult instar, when the laboratory population was quite old. The reason for the second peak is unexplained. Of the original 31 females, there were 11 still living and reproducing at the time of the second peak (Table 5). An examination of fecundity records for each of the original 31 females gives no indication that the peak was an artifact due to the dying off of less reproductive females, which, had this been the case, would have negatively influenced the mean brood size for a particular instar. The peak was real in the sense that the surviving females had larger individual broods at this time. The experiment at 20°C extended over 15 weeks and, although there was no change in "crowding" since the females were cultured individually, the culture medium was changed every other day. Perhaps, in some way unknown to us, the constituents of the culture medium varied at the time of the second peak, changing the food level and resulting in increased fecundity.

Table 5
Mortality of laboratory populations at 20°C and 25°C used to estimate mean number of young per brood (Figure 9)

Adult instar number	Number of survivors
20°C population	
1	31
2	30
3–9	29
10	28
11–12	27
13–14	25
15	23
16–17	20
18–20	19
21	18
22–24	17
25	16
26–27	15
28	14
29–30	12
31–32	11
33	10
34	8
35	4
36	2
37	1
38	0
25°C population	
1–15	8
16–17	6
18	5
19–20	4
21	3
22	3
23	0

The mean total young production per female (including all females, not just the mean total young of surviving females) at 25°C and 20°C was 234 and 378 respectively—the mean number of young per brood being 12.5 at 25°C and 16.3 at 20°C. Females at 25°C produced fewer young per brood than females at 20°C and because they did not live as long as those at 20°C, their mean total egg production was considerably less than that for the 20°C females.

Broods occurred on the average every 2.7 days at 20°C and 18.1 days at 5°C (Fig. 10). Low temperature considerably delayed the onset of reproduction. At 20°C, the females, on the average, released their first brood seven days after being released from the brood chamber themselves; at 5°C the average time for this process was 64 days. Average total young production per unit time was, of course, greater at 20°C than at 5°C. For example, females 110 days old at 20°C had an average total young production of 378. For the same unit of time but at 5°C, average total young production was 67 for those females primiparous in the sixth instar, 44 for those primiparous in the seventh instar, and 34 for females primiparous in the eighth instar. These values for 5°C females also suggest that at the same temperature

there is possibly greater total production for females becoming primiparous in early instars.

In short, 20°C seems to be the optimum temperature (of the three tested) for young production. At 5°C, because of less frequent molts, young production per unit time was considerably less than at 20°C. At 25°C, broods per unit time were not recorded, but the longevity data for 25°C females (Fig. 1, p.28) indicate only slightly more instars per unit time (2.3 days per instar) than for those at 20°C. And, as indicated above, 25°C females produced fewer young per brood and did not live as long as 20°C females.

Sizes of females in first adult instar and number and size of young produced

There were variations in the number of young produced in the first adult instar and these variations were positively correlated with the size of the female in the first adult instar (Table 6). The correlation coefficient for the 55 females primiparous in the fifth instar at 20°C was 0.732 and is significant at the 99 per cent level. The correlation holds for other primiparous instars: for 27 females primiparous in the sixth instar (20°C) and 28 females primiparous in either the fifth or sixth instar (25°C) the correlation coefficients were 0.866 and 0.653 respectively, both being significant at the 99 per cent level.

Not only do larger females produce more young per brood in the first adult instar than smaller females, they also produce larger young. The correlation coefficient between the size of females in the first adult instar and the size of young produced by these females was 0.685 and is significant at the 99 per cent level.

There was a tendency for the size of the young to increase with increasing age (in instars) of the female (Fig. 11). This would be expected since the older females are larger. However this relationship did not hold for the one female that was followed through 34 instars. For this female, there was a sharp decline in average size of young after the twenty-third brood (twenty-seventh instar), even though the female continued to increase slightly in total length during its life-span.

Table 6
Female size in first adult instar, and number and size of their young in first adult instar*

Number of females	Mean length (mm) of females	Mean number of young	Mean length (mm) of young
1	1.44	3.0	0.55
2	1.56	5.5	0.54
1	1.57	4.0	0.57
1	1.59	8.0	0.53
1	1.60	8.0	0.54
3	1.63	6.7	0.55
3	1.66	4.3	0.61
1	1.68	2.0	0.62
1	1.69	3.0	0.60
1	1.70	5.0	0.57
6	1.72	4.7	0.60
1	1.73	4.0	0.59
2	1.76	4.5	0.62
5	1.79	5.8	0.59
1	1.80	4.0	0.63
1	1.81	6.0	0.62
7	1.82	5.6	0.59
1	1.83	5.0	0.61
5	1.85	5.0	0.62
1	1.88	7.0	0.62
1	1.89	9.0	0.56
1	1.93	10.0	0.59
2	1.98	9.5	0.59
1	1.99	9.0	0.58
1	2.20	8.0	0.67
1	2.21	8.0	0.67
2	2.24	11.0	0.66
1	2.28	14.0	0.65

*Based on 55 females primiparous in the fifth instar at 20°C.

Production of sexual eggs

In the laboratory, *D. schødleri* produced ephippia only at low temperatures; seven females produced sexual eggs at 5°C without the presence of males. These ephippial females were followed through a varying number of instars (Table 7). Four females, in addition to producing sexual eggs, also produced at least one brood of parthenogenetic eggs (all of which subsequently developed into females), while the other three females produced only sexual eggs. The unfertilized sexual eggs were followed for two months at 20°C, but they did not hatch. All seven females had a sterile instar immedi-

Table 7
Reproductive features of seven females that produced at least one ephippium at 5°C*

	Instars						
Animals	6	7	8	9	10	11	12
1	8	E	0	E	0	0	–
2		13	17	17	E	dead	
3		10	E	0	17	–	–
4		10	E	0	5	–	–
5		E	0	E	dead		
6		E	0	E	dead		
7		E	0	E	0	E	0

0 A sterile instar: neither sexual nor parthenogenetic eggs were produced.
E The production of an ephippium and ephippial eggs.
dead The death of the mother animal.
– No further observations.

*The numbers shown in each column represent the number of parthenogenetic young produced in the indicated instar.

ately following the ephippial instar. This is different from observations of other workers, e.g. Berg (1931), where no sterile instars were found, the daphnids producing either sexual or parthenogenetic eggs in the instar immediately following the ephippial instar.

In short, in the laboratory, individual *D. schødleri* producing sexual eggs may or may not also produce parthenogenetic eggs sometime during the life-span; even for those ephippial females that do subsequently (or did previously) produce parthenogenetic eggs, there is no strict alteration of the two types of reproduction in the sense of being predictable. The significance of the sterile instar is unexplained.

Since, for many aspects of Daphnia's biology, it is desirable to know if the females are about to produce or have in the past produced ephippia, we include here the description of ephippial formation for *D. schødleri*. Females that are to produce sexual eggs (and an ephippium) in the next instar usually can be recognized by the presence of a compact dark mass of small fat globules in the ovaries. The mass at first is very small and consists of closely packed small fat globules surrounded by larger fat globules. Near the end of the pre-ephippial instar, the compact dark mass has grown quite large, and it occupies nearly all of the ovary. The female soon undergoes ecdysis and the new exoskeleton of the female, which is now in the ephippial instar, has an indention on the dorsal margin at the head-carapace junction (Fig. 12A). The dorsal part of the carapace, which will eventually develop into the ephippium, now is light brown or gray in color, and it is separated from the other part of the carapace by an irregular line. Very shortly, two fully developed sexual eggs are deposited into the modified dorsal part of the carapace (Fig. 12B). The modified dorsal part of the carapace now becomes darker and is gradually pushed upwards (Fig. 12C). Its separation from the remainder of the carapace becomes evident and eventually the modified dorsal part is completely freed, by the mechanics of molting, from the rest of the carapace. The female, after molting and discarding the ephippium, still possesses an indention on the dorsal margin (Fig. 12D).

Table 8
Vital statistic properties of *D. schødleri*'s laboratory populations, and vital statistics of other species as gathered from selected reports

	D. schødleri, present study	Other studies
Number of preadult instars	4–7 (♀♀), 3–4 (♂♂)	4 (♀♀) *D. galeata mendotae*[1], *D. laevis*[2]; 3–5 (♀♀)*D. obstusa*[3]; 4–5 (♀♀) *D. pulex*[4], *D. curvirostris*[3], *D. schødleri*[5]; 4–6 (♀♀) *D. thomsoni*[3]; 5–6 (♀♀) *D. atkinsoni*[3]; 4–8 (♀♀) *D. magna*[6&7]
Maximum longevity	28 instars, 65 days (♀♀, 25 C) 41 instars, 113 days (♀♀, 20 C) 26 instars, 68 days (♂♂,25 C)	22 instars, 54 days (♀♀ *D. magna*[7], 25 C); 202 days (8 C), 150 days (10 C), 99 days (18 C), and 57 days (28 C)(♀♀ *D. magna*[8]); 26 instars, 149 days (♀♀ *D. schødleri*[5], 16 C); 179 days (8 C), 150 days (10 C), 92 days (18 C), and 46 days (28 C) (♂♂ *D. magna*[8])
Greatest absolute growth increment	Between adolescent and primiparous instar (♀♀); between preadolescent and adolescent instar (♂♂).	Between adolescent and primiparous instar (♀♀ *D. pulex*[4]) (♀♀ *D. magna*[8]); between preadolescent and adolescent instar ♀♀ *D. curvirostris* and others[3]; species with both the above and even prior to preadolescent instar[3].
Greatest relative growth increment	Prior to preadolescent instar (♀♀); between preadolescent and adolescent instar (♂♂).	
Relative growth	Carapace length vs. total length, k = 1.059 (♀♀), 1.098 (♂♂); height vs. total length, k = 1.079 (♀♀), 0.972 (♂♂).	Height vs. total length, k = 1.09, 1.03, and 0.315, for three growth stanzas, *D. pulex*[9]; k = 1.13 and 1.05 for two growth stanzas, *D. magna*[9].
Instar with greatest relative variability	Carapace length: fourth instar (♀♀), second instar (♂♂); height: third instar (♀♀), second instar (♂♂); total length: third instar (♀♀), second instar (♂♂).	
Adult instar with largest number of young or eggs	Fourth (25 C), eighth and thirty-first (20 C)	Seventh *D. magna*[10], fifth *D. magna*[7], sixth *D. laevis*[2]
Maximum number of young or eggs per brood	24 (25 C), 39 (20 C)	105, *D. magna*[10]; 36, *D. laevis*[2]
Mean number of young or eggs per brood	12.5 (25 C), 16.3 (20 C)	6.3, *D. schødleri*[5] (16 C); 5.8–23.1, *D. laevis*[2] (depending on the medium); 6.7–25.9, *D. laevis*[2] (depending on the clone).

	D. schødleri, present study	Other studies
Mean total number of young or eggs for 110-day period	48 (5 C), 378 (20 C), 234 (25 C)	36 (8 C), 49 (18 C), 15 (28 C), *D. magna*[6]; 150 (25 C), *D. magna*[7]; 138 (16 C), *D. schødleri*[5]; 1,072, *D. magna*[10] (one individual)

1 Hall 1962.
2 Ingle et al. 1937.
3 Green 1956.
4 Anderson, Lumer and Zupancic 1937.
5 LeSuer 1959.
6 Anderson 1932.
7 Anderson and Jenkins 1942.
8 MacArthur and Baillie 1929.
9 Hersh and Anderson 1941.
10 Kerhervé 1927 (cited in Hutchinson 1967:581).

Discussion

Table 8 summarizes the vital statistic properties of the *D. schødleri* laboratory populations and also summarizes values for other species, as gathered from selected reports. One must use caution when comparing interspecific values of different studies, even at the same temperature, because of the culture medium's food level, which in most studies is unquantified, and which can affect most of the vital properties. Even when the laboratory culture conditions are adequately quantified, equating laboratory food levels with those of the field is difficult; hence one must also use the utmost caution in associating laboratory life history phenomena with life history phenomena of field studies.

The instar in which the female becomes primiparous is important in influencing many other life history phenomena. For example, *D. schødleri* females becoming primiparous in early instars were generally smaller than females becoming sexually mature in later instars; large female neonates in the first instar became primiparous in earlier instars than did smaller neonates. At 5°C, females becoming primiparous in early instars produced more young during their life-span than did females becoming primiparous in late instars. Within a specific primiparous instar, the larger females produced more and larger young per clutch than did the smaller females. In short, by knowing the primiparous instar, much of the variability of life history properties can be accounted for. Determining and considering the primiparous instar should be as much a part of a controlled laboratory experiment as controlling the temperature or food level.

There are not many studies dealing with the biology of male *Daphnia*. We found that male *D. schødleri* differed from the females mainly in respect to various growth properties. The survivorship curves of males and females at the same temperature are similar, the males being as long-lived as the females and having about the same number of instars. But at the same temperature, females became primiparous from instars five to eight, while males became sexually mature in earlier instars, mainly in the fourth instar. These differences in time of sexual maturity influenced most of the other growth parameters studied and compared. The absolute growth curves for height, carapace length, and total length of females had, because of the later onset of sexual maturity, a later inflexion point; also the growth curves (in instars) of females were more typically sigmoid than those of males. Related to this and also influenced by the onset of sexual maturity, the absolute and relative growth increment curves of males and females were different. For males, both the greatest absolute and relative growth increments came at the molt between the preadolescent and adolescent instars; for females, the greatest absolute increment was between the adolescent and primiparous instars, and the largest relative growth increment came even before the preadolescent instar.

Pertaining to relative growth per se, the length of the male's carapace increased relatively more rapidly than did total length, more so than the same phenomenon in

females. But height of males, as might be expected considering the shape and smaller adult size of males, increased relatively less rapidly than did total length; in contrast the height of females increased relatively more rapidly than did total length. As would be expected considering the nature of coefficient of variation values, the relative variability of the values of the three linear dimensions — carapace length, height, and total length — was much greater for both males and females during the preadult instars. The relative variablility of the females' dimensions extended over a longer time (in instars) and this is probably accounted for by the later onset of sexual maturity of females. In the middle and late adult instars, the linear dimensions' relative variability was quite similar for both sexes.

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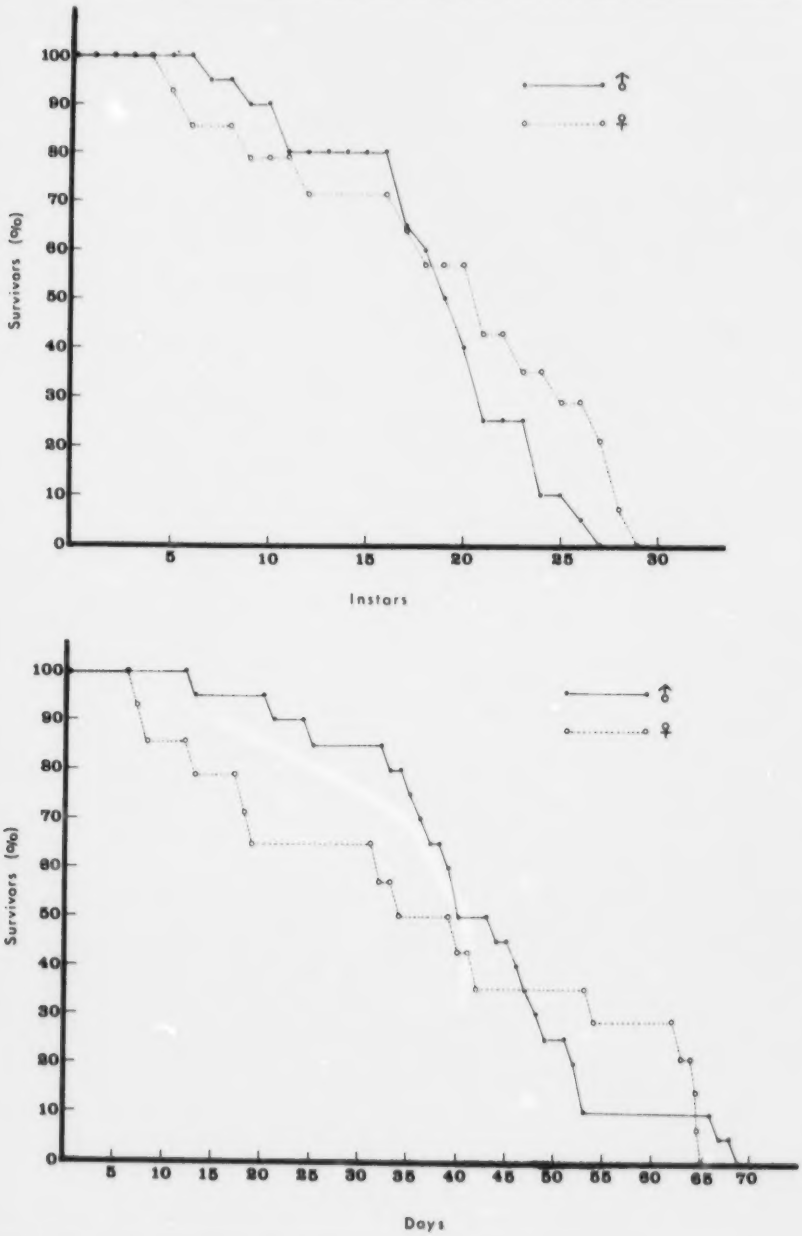

Figure 1
Survival curves for male and female *D. schødleri*, in instars and days. Data based on 20 males and 14 females at 25°C.

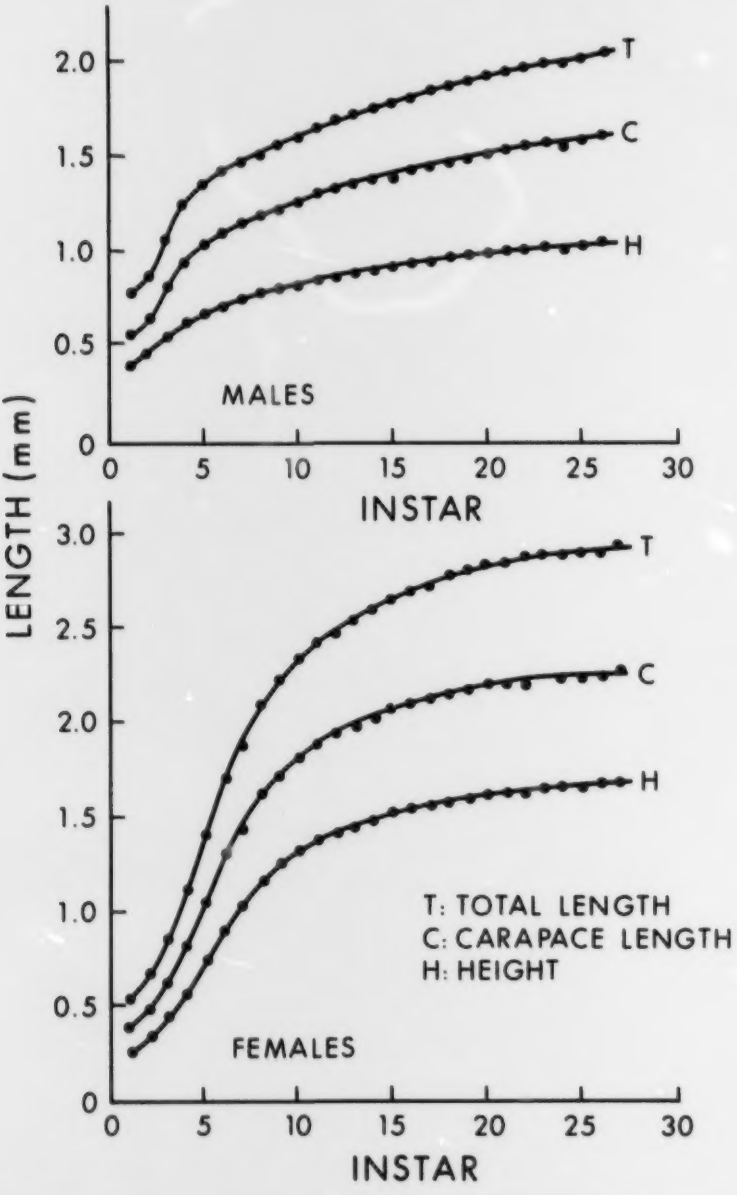

Figure 2
Mean growth curves based on data of eight females that were primiparous in the sixth instar and lived for at least 20 instars at 25°C and eight male *D. schødleri* that lived for 18 or more instars at 25°C.

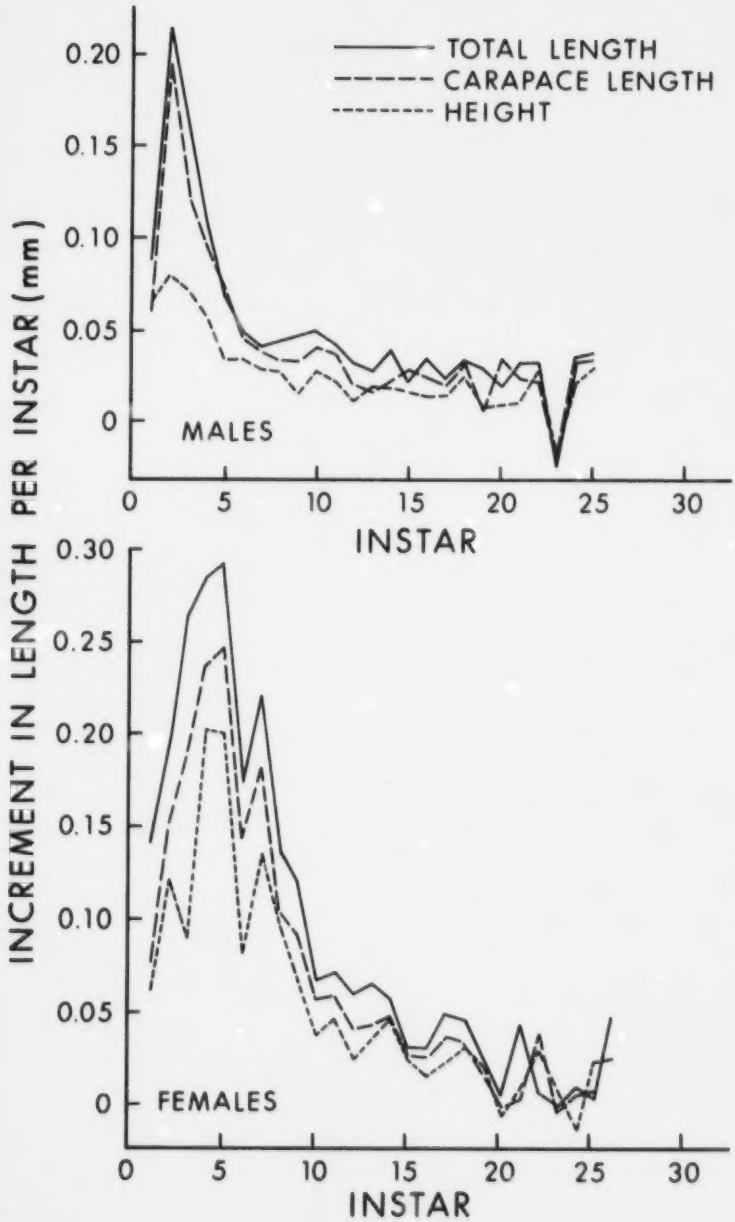

Figure 3
Absolute growth increment curves for the males and females of Figure 2. The increment values of a particular instar represent the absolute increase in size between that instar and the instar to follow, e.g. the value at instar 5 represents the increase between 5 and 6.

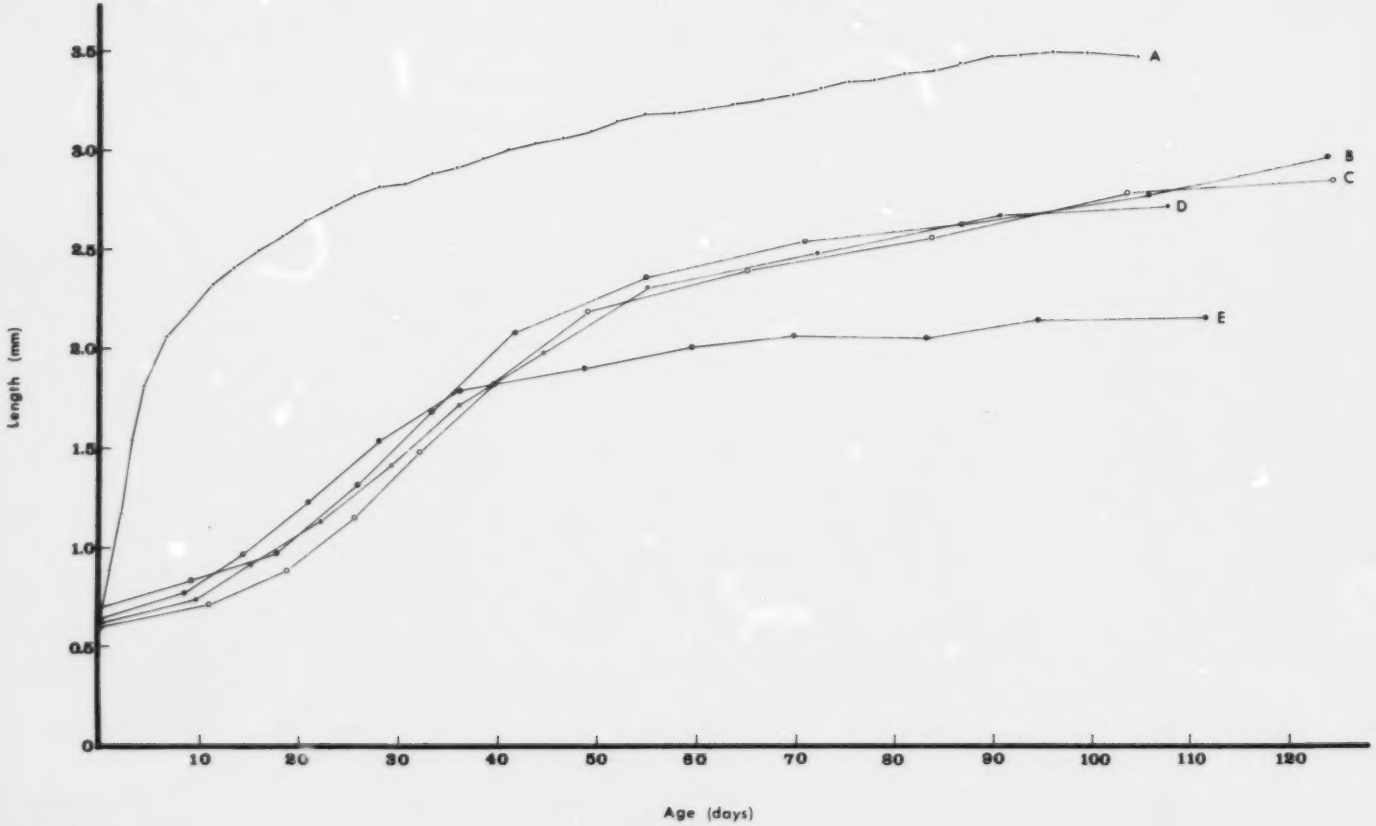

Figure 4
Growth curves in days of female *D. schødleri* at 20°C and 5°C. The dots indicate instars.
A, 31 females primiparous in the fifth instar at 20°C;
B, six females primiparous in the sixth instar at 5°C;
C, 10 females primiparous in the seventh instar at 5°C;
D, two females primiparous in the eighth instar at 5°C;
E, three females primiparous in the seventh instar at 5°C, but producing ephippia instead of parthenogenetic eggs.

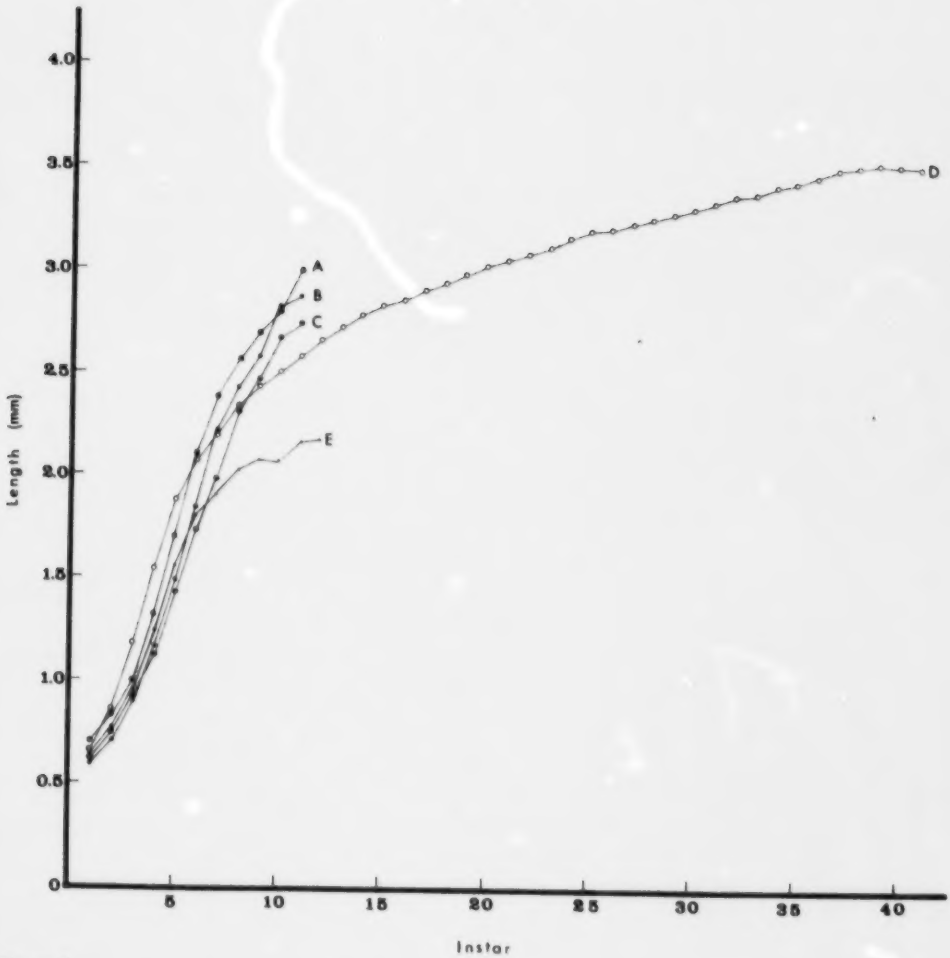

Figure 5
Growth curves in instars of female *D. schødleri* at 20°C and 5°C.
A, six females primiparous in the sixth instar at 5°C;
B, 10 females primiparous in the seventh instar at 5°C;
C, two females primiparous in the eighth instar at 5°C;
D, 31 females primiparous in the fifth instar at 20°C;
E, three females primiparous in the seventh instar at 5°C, but producing ephippia instead of parthenogenetic eggs.

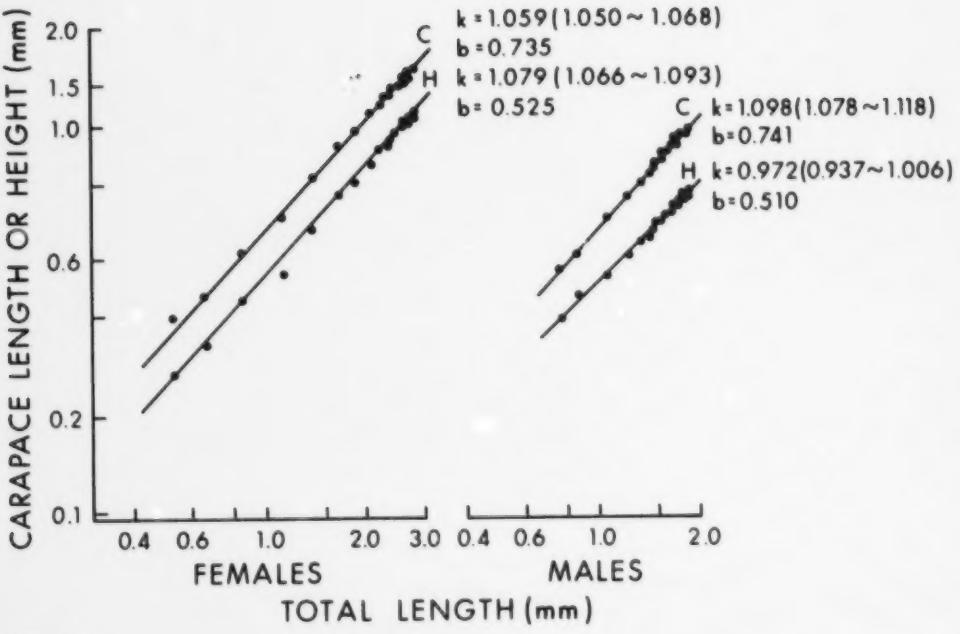

Figure 6
Scatter diagrams of mean carapace length and height plotted against mean total length for the first 18 instars of the eight females and the eight males of Figure 2; both variates plotted logarithmically. The allometric constants b and k, and k's 95 per cent confidence intervals (in parathesis) are shown for each relationship.
C = carapace length, H = height.

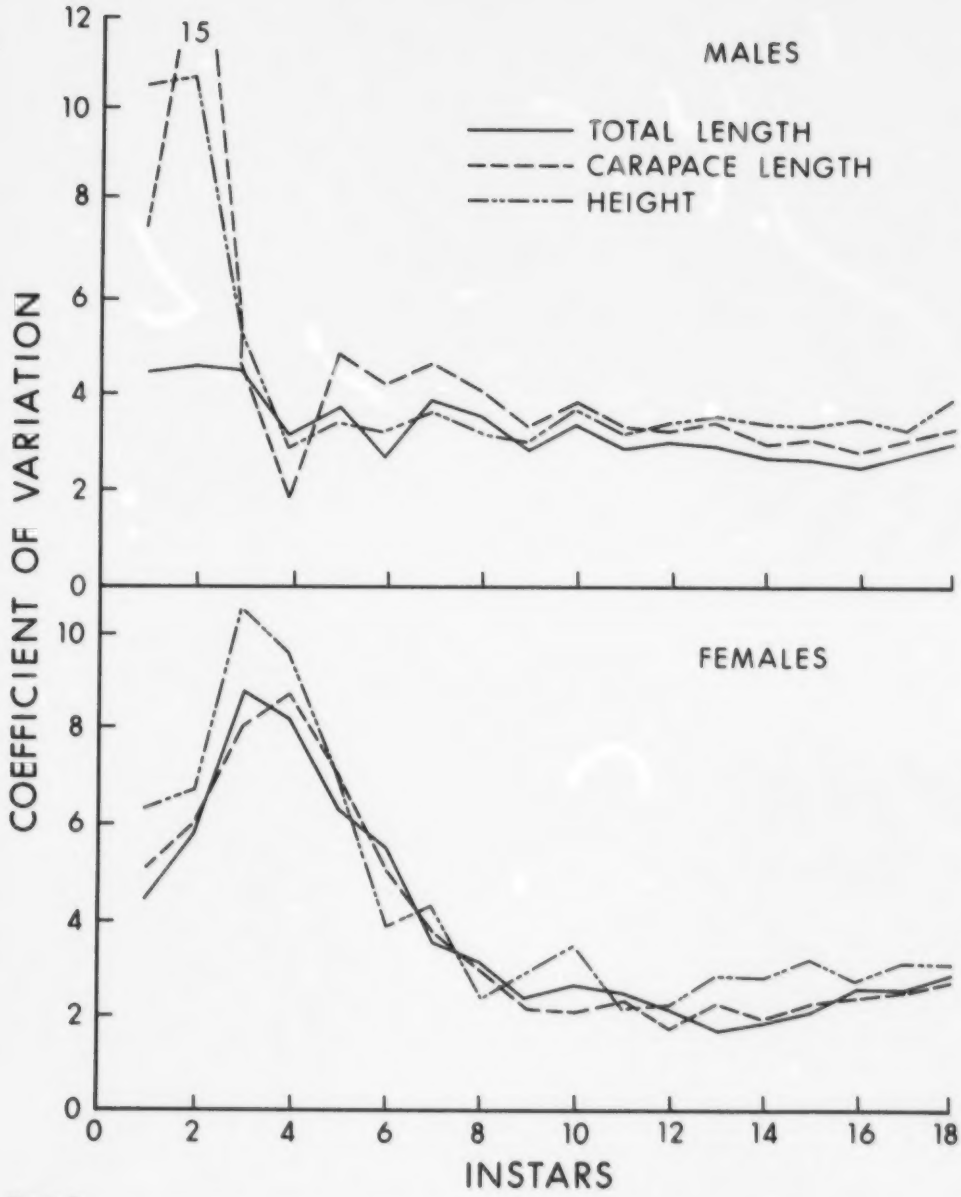

Figure 7
The coefficients of variation (= 100 times standard deviation divided by the means) for each linear dimension for the first 18 instars of the eight females and the eight males of Figure 2.

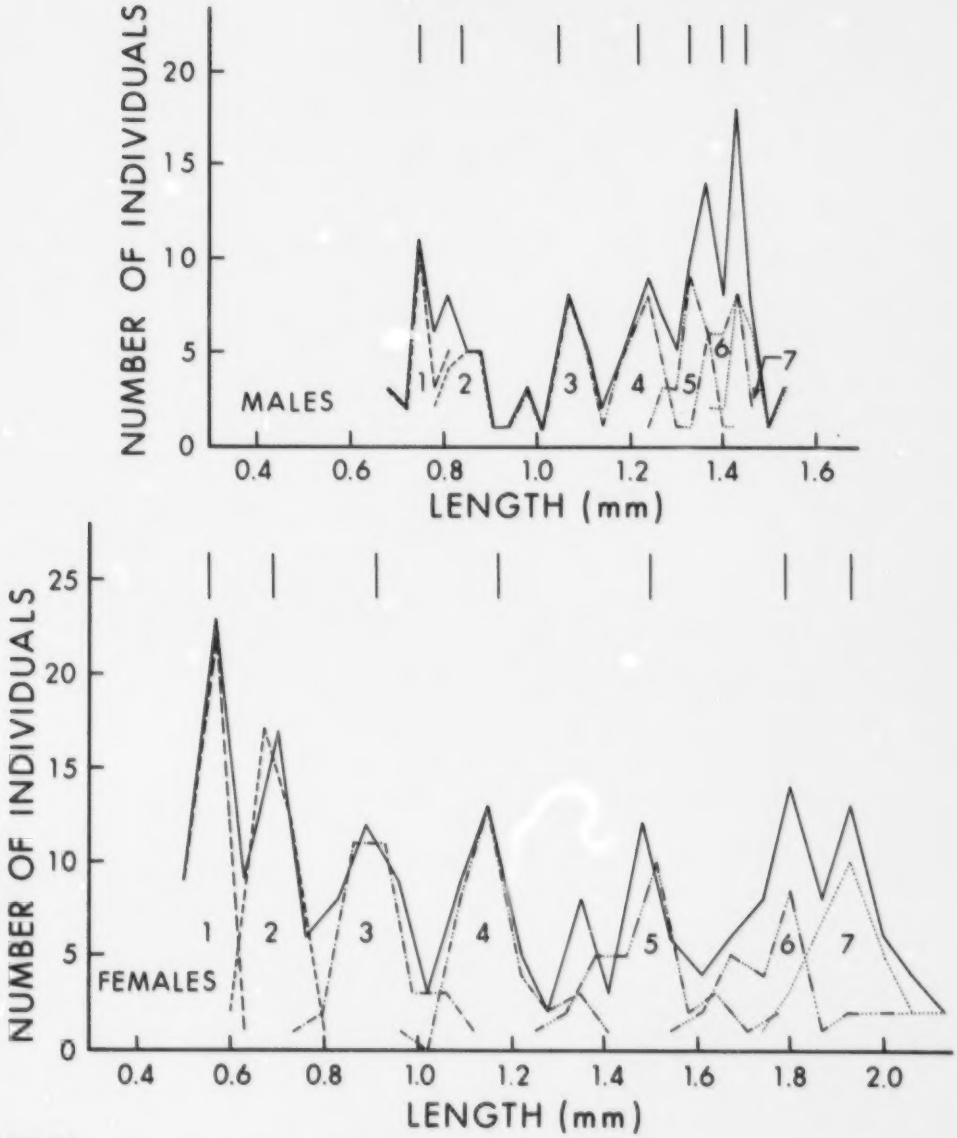

Figure 8
Size–frequency distributions during the first seven instars of 24 males and 32 females reared at 25°C. Broken lines designate the discrete instars. The solid line is a composite curve for all instars (see text for further explanation). The vertical bars near the upper edge of the figure represent the mean total lengths for each of the first seven discrete instars.

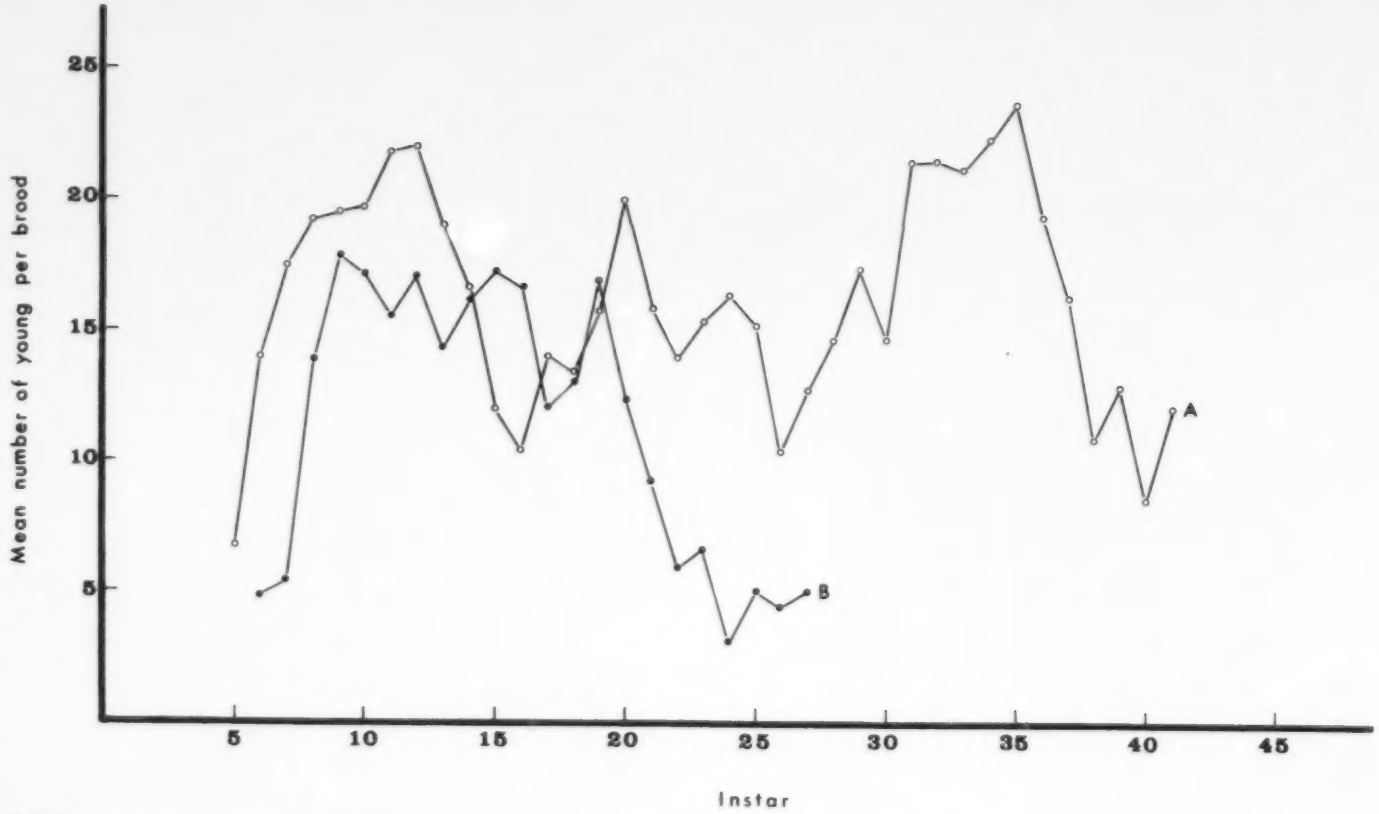

Figure 9
Mean number of young produced during each adult instar.
A, based initially on 31 females primiparous in the fifth instar at 20°C;
B, initially eight females primiparous in the sixth instar at 25°C.
See Table 5 for mortality during each population's life-span.

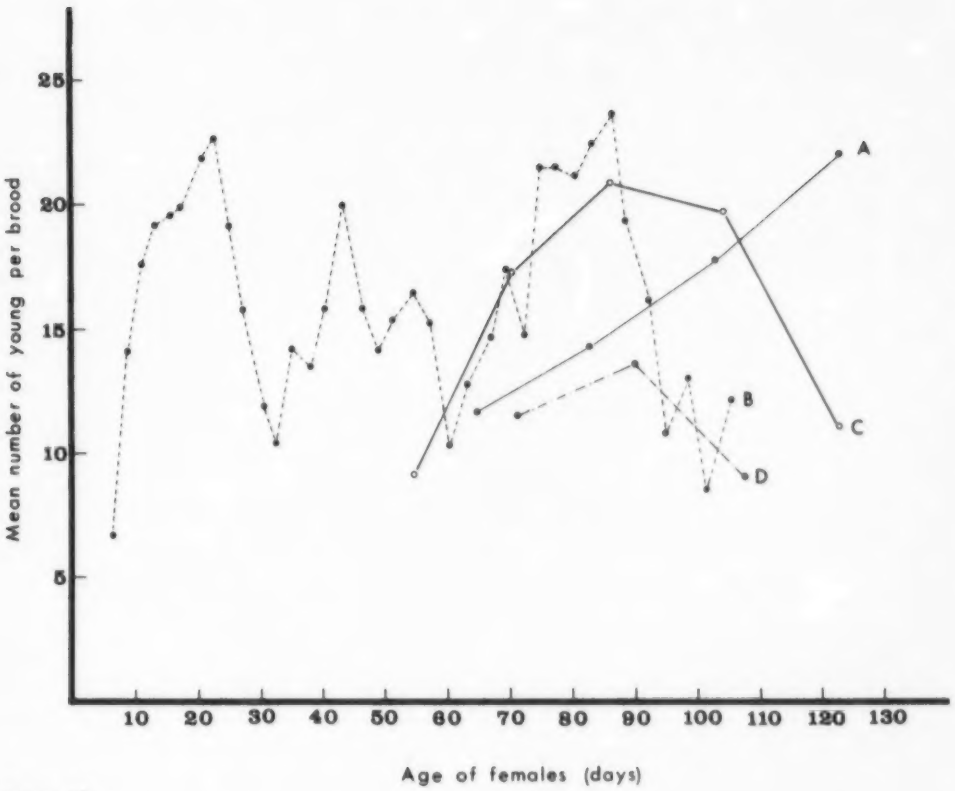

Figure 10
Mean brood size in relation to age (in days) of females.
A, based initially on females primiparous in the seventh instar at 5°C;
B, data based initially on females primiparous in the fifth instar at 20°C;
C, data based initially on females primiparous in the sixth instar at 5°C;
D, data based initially on females primiparous in the eighth instar at 5°C.

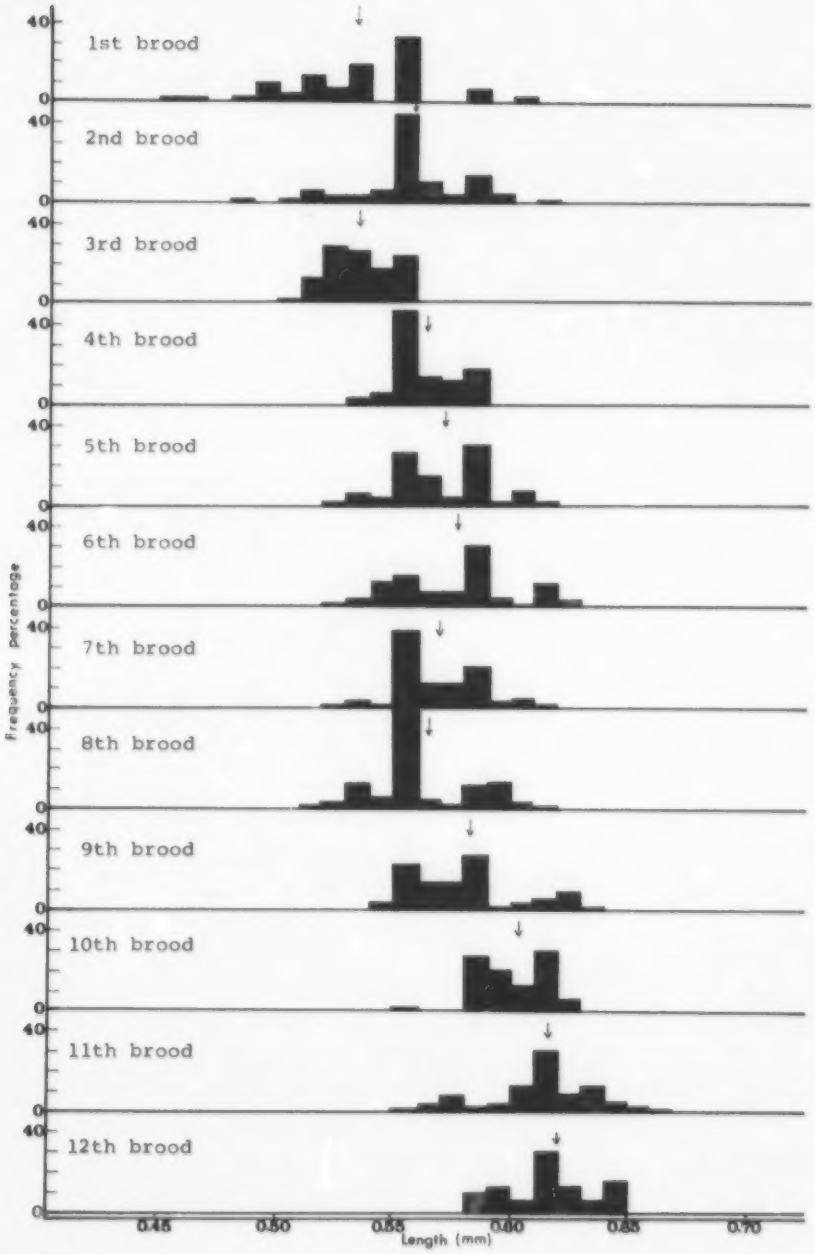

Figure 11
Length of liberated young in different broods, shown as percentage of size–frequency distribution; based on six females for the first 11 broods and three females for the twelfth brood, all primiparous in the fifth instar at 20°C. The arrows indicate the mean length of the young.

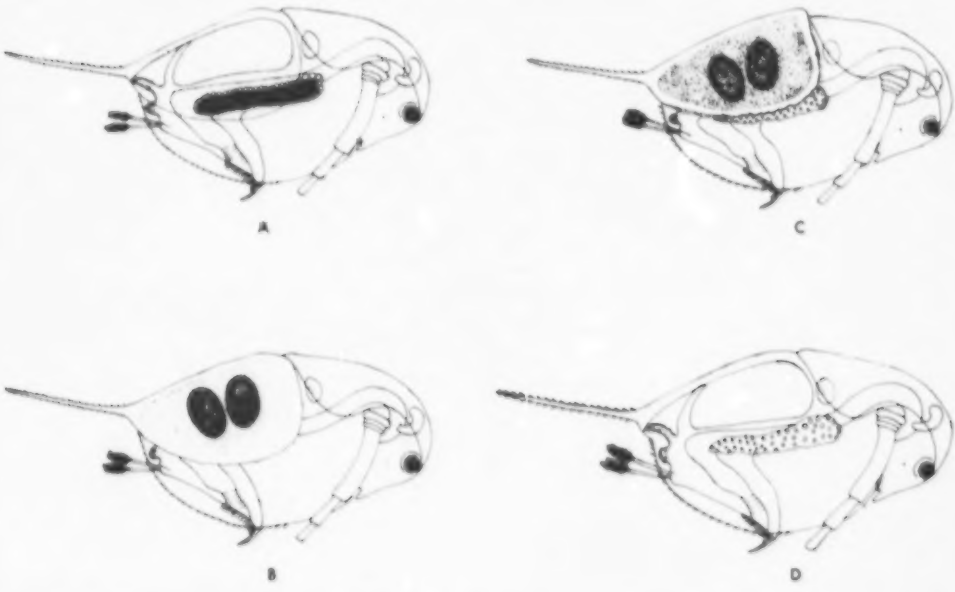

Figure 12
Formation of an ephippium in *D. schødleri*.
A, female at the beginning of ephippial instar, showing fully grown sexual egg in ovary and the modified dorsal part of carapace;
B, the middle of ephippial instar, showing two sexual eggs, which have been deposited into modified dorsal part of carapace;
C, near the end of the ephippial instar;
D, female in the sterile instar, after molting and discarding ephippium.

Part III Periodicity, Reproduction, and Growth of *D. schødleri* in the Lake

Abstract

Daphnia schødleri Sars from Big Island Lake overwintered in the resting egg stage. The *ex ephippio* generation appeared in late spring and by May parthenogenetic reproduction was taking place. There were two periods of sexual reproduction — a major period in June and July, at which time the population was exhibiting maximum numbers, and a minor period in September. The entire first period of sexual reproduction is accounted for by the *ex ephippio* generation, the ephippial females of the second period were probably of the third generation. There were, in all likelihood, between four and five reproducing generations (either parthenogenetic or sexual) during the open season. According to Hutchinson's terminology (1967), *D. schødleri* in Big Island Lake would be an aestival, more or less monoacmic, dicyclic species. Production of parthenogenetic eggs varied seasonally. The average number of eggs per brood diminished just before the beginning of the sexual periods, but the decrease in brood size was not due to a decrease in the average size of egg-bearing females at this time. The overall average brood size for the entire study period was 7.96 eggs, which is considerably lower than the average brood size of the laboratory populations. The size of resting eggs varied with size of the ephippial females; generally, ephippial females were smaller than parthenogenetic females.

Introduction

Part III of the study of *Daphnia schødleri* Sars is concerned with *D. schødleri*'s biology in Big Island Lake, Alberta. Although Parts I and II dealing with the embryology and vital statistic properties of laboratory populations have been reported first, all three studies were carried out more or less concurrently. For this reason, it was not possible to design the field study to take advantage of what was learned from the laboratory populations. Nevertheless, the laboratory data were important for intepreting aspects of *D. schødleri*'s biology from the lake.

Materials and Methods

D. schødleri was studied in Big Island Lake from 13 June 1966 to 3 July 1967. Twenty-four samples were taken from the limnetic region during this period, using a plankton net with a number 20 mesh size and 12.5 cm diameter opening. Plankton samples were preserved in five per cent formalin. In the laboratory each sample was diluted to a known volume (usually 100 to 200 ml), depending upon the number of plankters present in the sample. *D. schødleri* and other plankton of several 1 ml subsamples were counted in a Sedgwick-Rafter cell under low power (40x) of a microscope. Length measurements were made from the top of the head to the base of the spine; this was designated as body length.

To facilitate the analysis of the population composition of *D. schødleri*, individuals in each sample were grouped into five categories as suggested by Green (1955):

1) Females with parthenogenetic eggs or embryos in the brood chamber, or females with large ovaries indicating that eggs were about to be laid.

2) Females of mature size (over 54.7 micrometer units = 1.78 mm) but without eggs or large ovaries, and possessing the long abdominal process by which eggs are retained in the brood chamber.

3) Females with ephippia, or females with the carapace showing signs of ephippial formation and the corresponding appearance of sexual eggs in the ovary; females with sexual eggs in the ovary, but not showing any signs of ephippial formation. These ovaries are composed of a compact dark mass, representing the developing sexual eggs.

4) Immature females. These are smaller than mature females (i.e. smaller than 1.78 mm) and do not yet have the long abdominal process that retains eggs in the brood chamber.

5) Males — either immature or mature specimens.

The carapaces of category 1 animals were opened with fine needles, and the number of eggs or embryos in the brood chambers was counted under low power of a dissecting microscope. The term "egg number" is used irrespective of whether eggs or embryos were counted. The mean egg number was calculated from a sample of at least 25 females having eggs in their brood chambers.

To estimate the volume of parthenogenetic eggs (egg size), samples of at least 50 eggs were dissected out of the brood chambers of females of various sizes, and measurements were made with the eggs covered by a film of water on a slide. The volume of each egg was then calculated using the formula:

$V = 1/6\pi gs^2$

where g is the largest diameter and s is the least diameter of the egg. This formula was used by Green (1956) to calculate the volume of cladoceran eggs, which are not true spheres. Volumes were determined only for eggs at late Stage I or early Stage II of embryonic development, developing embryos beyond early Stage II being rejected (see Part I for explanation of stages). Since newly deposited eggs tend to swell when first laid into the brood chamber, these also were rejected. The same methods were used to measure the length of ephippial females and to calculate the volume of sexual eggs.

Table 1
Limnetic zooplankton found in Big Island Lake, other than Protozoa

	1966												1967							
Samples collected	13 June	22 June	29 June	8 July	13 July	27 July	3 Aug.	17 Aug.	26 Aug.	16 Oct.	27 Nov.	18 Dec.	5 Jan.	19 Mar.	9 Apr.	13 May	27 May	3 June	13 June	24 June
Daphnia schødleri Sars	x	x	x	x	x	x	x	x	x	x							x	x	x	x
Diaphanosoma leuchtenbergianum Fischer			x	x	x	x	x	x	x	x							x	x	x	x
Bosmina coregoni Baird	x		x	x		x	x	x		x	x	x					x		x	
Chydorus sp.										x	x						x			
Ceriodaphnia sp.	x				x															
Cyclops varicans rubellus Lilljeborg	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x
Cyclops bicuspidatus thomasi Forbes	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x
Macrocyclops albidus (Jurine)	x	x	x	x	x	x	x	x	x	x			x	x		x	x	x	x	x
Eucyclops agilis (Koch)	x	x		x	x	x			x	x	x	x		x		x	x	x	x	x
Diaptomus siciloides Lilljeborg	x	x	x	x	x	x	x	x	x	x	x							x	x	x
Nauplii	x	x	x	x	x	x	x	x	x	x	x					x	x	x	x	x
Keratella cochlearis (Gosse)	x	x	x	x	x	x	x	x	x	x	x					x	x	x	x	x
Keratella quadrata (O.F.M.)	x	x	x	x		x	x	x	x	x	x					x	x	x	x	x
Brachionus sp.	x		x	x		x	x	x	x								x	x	x	x
Rotaria neptunia (Ehrenberg)	x		x	x	x	x	x	x	x		x	x	x	x	x	x	x	x	x	x
Filinia longiseta (Ehrenberg)	x	x	x	x	x	x	x	x	x	x	x						x	x	x	x
Asplanchna sp.	x	x														x	x			x
Polyarthra sp.	x		x															x		x
Trichocerca sp.			x	x	x	x														
Dipleuchlanis sp.	x															x				
Lepadella sp.																x				

x Present in sample.

Description of the Study Area

Big Island Lake is a shallow, unstratified, eutrophic lake located about 17 miles southeast of Edmonton, Alberta. It is one of several small kettle lakes, remnants of the Wisconsin glaciation, characteristic of the northern prairies. Eutrophication and senescence in a group of these lakes were described by Kerekes and Nursall (1966), and Nursall (1969). Big Island Lake has a surface area of about 121 hectares (300 acres), with a maximum depth of 2.5 meters; most of the lake is less than two meters in depth. An island, located in the middle of the lake, covers an area of nearly five hectares.

Figure 1 shows water temperatures and dissolved oxygen values recorded during the study period. From November 1966 through April 1967, the lake was completely ice-covered, the average water temperature during this period being 1.5°C. Winter stagnation occurred from January through April; during this period there was no detectable dissolved oxygen, and hydrogen sulfide was present. The ice started breaking up in early May 1967.

There are no fish in the lake. The dominant macro-invertebrates are the amphipods *Gammarus lacustris* Sars and *Hyalella azteca* (Saussure). The population ecology of *G. lacustris* in Big Island Lake was described by Menon (1969). Extensive algae blooms occurred sporadically during the summers of 1966 and 1967. Major bloom organisms were the blue-greens *Microcystis flos-aquae* Kirchn., *M. aeruginosa* Kuetz and *Anabaena flos-aquae* Breb. Other important algae were *Pediastrum boryanum* Menegh., *P. duplex* Meyen and species of *Scenedesmus* and *Staurastrum*.

Zooplankton of Big Island Lake

Table 1 lists the limnetic zooplankters, other than Protozoa, collected during the 14-month study period. The only zooplankters present in the lake throughout the entire winter were the cyclopoids *C. varicans rubellus* and *C. bicuspidatus thomasi*, and the rotifer *Rotaria neptunia*.

Seasonal changes in the population densities of the major zooplankters are shown in Figure 2. Population densities of the copepods include both adults and copepodites. The seasonal curve for Cyclopoida represents all species of this suborder found in the lake, *C. varicans rubellus* and *C. bicuspidatus thomasi* being the most abundant species. The 1966 cyclopoid population reached its peak in November. From December 1966 through 9 April 1967, all cyclopoids consisted of late copepodites and a few adult females; apparently reproduction does not take place during the winter months in Big Island Lake. In 1967, females with egg sacs were first collected on 13 May; males and a few nauplii were also present at this time.

Diaptomus siciloides reproduced almost continuously during the summer months of 1966, females carrying egg sacs or spermatophores being collected from June through mid-October 1966. The population reached its greatest density in late August; by 27 November, only a few *D. siciloides* were found in the lake. These specimens were adult males and females, but none of the females was carrying egg sacs or spermatophores; also nauplii were absent at this time. By 18 December, *D. siciloides* had completely disappeared from the lake, and was not collected again until the next spring.

Diaphanosoma leuchtenbergianum reached maximum numbers earlier in the ice-free season than did the copepods. Males, and females carrying resting eggs, were first collected in August 1966; on 16 October, females with resting eggs were still present in the lake. In 1967, *Diaphanosoma* first appeared in late May, at which time the entire population consisted of young females. Adult females carrying eggs and embryos were first collected on 13 June 1967.

Table 2
Seasonal changes in percentage composition of life-cycle stages of *D. schødleri* in Big Island Lake, June 1966 to July 1967

Date	Females with parthenogenetic eggs	Females of mature size without eggs	Immature females	Ephippial females	Males	Number of individuals counted
1966						
May 3	0	0	0	0	0	0
June 13	6.3	2.6	91.1	0	0	304
22	13.2	14.1	29.2	42.9	0.6	319
29	9.3	21.8	28.0	40.9	0	193
July 8	7.4	0.7	87.4	4.1	0.4	269
13	11.4	2.2	85.9	0.5	0	185
19	44.2	10.9	41.0	3.9	0	129
27	17.4	2.9	78.3	1.4	0	69
Aug. 3	1.9	0	98.1	0	0	103
17	19.4	9.7	70.9	0	0	31
26	20.0	20.0	60.0	0	0	5
Sept. 28	15.0	18.0	63.0	3.7	0.3	85
Oct. 16	0	33.3	50.0	0	16.7	6
Nov. 27	0	0	0	0	0	0
Dec. 18	0	0	0	0	0	0
1967						
Jan. 5	0	0	0	0	0	0
Mar. 19	0	0	0	0	0	0
Apr. 9	0	0	0	0	0	0
May 3	0	0	100.0	0	0	5
13	0	0	100.0	0	0	8
27	16.2	0.8	83.0	0	0	130
June 3	20.4	5.6	73.6	0	0.4	284
13	5.9	4.0	71.6	18.4	0.1	1287
24	13.5	11.0	56.3	18.9	0.3	318
July 3	7.6	5.5	75.9	7.2	3.8	237

Life History of *D. schødleri*

D. schødleri overwinters in Big Island Lake in the resting egg stage. Soon after the resting eggs hatched in the spring (May) of 1966, the population rapidly increased in number via a series of parthenogenetic generations, the *D. schødleri* population reaching its maximum density in June (Fig. 2). Parthenogenetic egg production started to decline at approximately the time maximum numbers were reached, and males and ephippial females appeared in the population (Table 2 and Fig. 3). There were two periods of sexual reproduction in 1966. The first started during the latter part of June and continued through July, lasting about five or six weeks. The second period occurred during September, at which time only a small number of ephippial females were collected. Between the two periods of sexual reproduction, the parthenogenetically reproducing population showed a slight increase in numbers. However, as the lake was sampled only once between 26 August and 16 October, the extent of this recovery and the exact length of the second period of sexual reproduction is not known. The population continued to decline after the second period of sexual reproduction, and *D. schødleri* completely disappeared from the lake in early November. According to the Hutchinson's terminology

Table 3
Seasonal variations in mean sizes of parthenogenetic and gamogenetic females, mean number of parthenogenetic eggs, and mean volume of sexual eggs

Date	Mean length of parthenogenetic females with eggs	Mean number of parthenogenetic eggs per female	Mean length of gamogenetic females	Mean volume of sexual eggs
	mm		mm	$mm^3 \times 10^3$
1966				
June 13	2.32	8.6		
22	2.70	5.9		
29	2.60	2.5	2.14	6.98
July 8	2.56	5.4	2.29	9.45
13	2.64	7.2	2.28	8.21
June 19	2.07	3.9	1.83	4.23
27	2.04	4.6	2.26	4.03
Aug. 3	2.03	6.8		
17	2.17	7.4		
26	2.15	11.0		
Sept. 28	2.32	3.9		
1967				
May 27	2.62	29.9		
June 3	2.30	13.1		
13	2.63	6.7	2.20	8.07
24	2.50	6.3	2.38	8.12
July 3	2.57	4.2	2.29	7.20

(1967), *D. schødleri* of Big Island Lake would be an aestival, more or less monoacmic, dicyclic species.

Female daphnids of mature size but without eggs are often considered to be old, sterile females, which have temporarily or permanently stopped producing eggs. In Big Island Lake, mature females without eggs made up a considerable part of the population in late June 1966 and again in the autumn (Table 2). In the laboratory (Part I), we found that females in late instars had a long barren period between releasing the brood and molting again (but after the molt another clutch of eggs was laid into the brood chamber), and hence what might, in analyzing field populations, be taken for either temporarily or permanently sterile females could, in fact, be old, slowly reproducing females. But we also determined in the laboratory (Part II) that all females producing sexual eggs had a sterile instar immediately following the ephippial instar. Assuming these phenomena also hold for the field population, we suggest that the relatively large percentage of mature females without eggs in late June were mainly females that had produced ephippia (the first period of sexual reproduction) and were in the sterile instar. The large percentage of mature females without eggs in autumn was probably due to both females being in the sterile instar following the ephippial instar (the second period of sexual reproduction) and also to a preponderance of old, slowly reproducing, but not sterile, females in the lake.

Also paralleling our laboratory observations, we found a positive correlation between the size (total length) of parthenogenetic females from Big Island Lake and the number of eggs in the brood chambers (Table 4). The degree of correlation between the size of females and the number of eggs in the brood chamber indicates a significant positive correlation (99 per cent level) for all sampling dates that were tested.

The size of sexual eggs was positively correlated with the body size of ephippial females carrying these eggs (Fig. 4). The

Table 4
Relationship between parthenogenetic egg production and body size of mature female *Daphnia schødleri* for different sampling dates

Date	Correlation Coefficient
July 13, 1966	0.602
July 19	0.740
July 27	0.865
May 27, 1967	0.916
June 3	0.610
June 13	0.852
June 24	0.934

correlation coefficient was 0.754 and is significant at the 99 per cent level. It is often stated that sexual eggs of *Daphnia* are larger than the parthenogenetic eggs (e.g. Banta et al. 1939; Lack 1954). For *D. schødleri* this is not the case, the sexual eggs being larger or smaller than parthenogenetic eggs, depending, at least in part, on the size of the females. Green (1956) found a similar relationship between sexual and parthenogenetic eggs of *D. magna*. For the field population, there was no significant correlation between the size of parthenogenetic eggs and the size of the females carrying these eggs.

Degenerate eggs were frequently observed in the brood chambers of *D. schødleri* females from Big Island Lake. These eggs were dark gray-brown and appeared to be disintegrating; normal eggs were blue-green or yellow-brown and were of a firm texture. Degenerate eggs were excluded from the egg counts and no attempt was made to calculate the percentage of degenerate eggs in relation to total eggs present. On 22 June 1966 and again on 13 June 1967, when algae blooms of *Microcystis* and *Anabaena* were extensive, a large proportion of parthenogenetic females (about 20 per cent of all mature females) was carrying disintegrating embryos. These embryos were found entangled in the *Microcystis* and *Anabaena* present in the brood chambers. Brooks (1946) suggests that egg degeneration in Daphnia is due to inadequate nutrition. Hall (1964) suggests that degenerate eggs may reflect a specific nutritional deficiency, a change in food level, temporary anoxia, or other conditions. In Big Island Lake, temporary anoxia, especially at night when the algae in the brood chamber are taking up oxygen, may be a major factor.

Interpretations of Length–Frequency Distributions

Because of environmental factors, especially nutrition, age of Daphnia from field populations cannot strictly be equated with size. Although both age and size are necessary to determine accurately factors affecting the growth of individuals, some understanding of the oscillatory nature of the various size (e.g. length) patterns of individuals comprising the population can be obtained by arbitrarily selecting size–class limits and following these size classes throughout the year. Also, by utilizing data available on population density (Fig. 2, p.50) and life history stages (Table 2, p.44) a more accurate interpretation of size–frequency distributions is possible.

Total length of at least 100 females for each sampling date was measured; in samples containing fewer than 100 females, all specimens were measured. The data were then grouped into histograms, each bar representing five eyepiece micrometer units, or 0.16 mm (Fig. 5). It was realized that these size–class limits were too wide to detect discrete instars (see Part II for the relation between discrete instars and size–class groupings). We determined from laboratory work that the mean size of first instar females was 18.5 micrometer units (0.60 mm) and the mean size of females in the first adult instar was 54.7 micrometer units (1.78 mm). In the field study, the size of the smallest parthenogenetic female with eggs was 50.0 units (1.63 mm), and the size of the smallest ephippial female was 54.5 units (1.77 mm). Therefore, individuals larger than 55 units were considered adults, and those which were 55 units and smaller were considered immature animals.

On 13 June 1966, the entire population was exclusively parthenogenetic and contained a large proportion of immature females (Fig. 5, and Table 2, p.44). Slobodkin (1954) has shown that in the early stages of population growth the few adult animals will have a high reproductive rate, resulting in a size–frequency distribution that is skewed towards the small end. For *D. schødleri*, this was apparently the situation on 13 June, resulting in a rapidly reproducing population containing a large proportion of small animals. On 29 June the size–frequency distribution had shifted to larger animals with most of the population consisting of adult females. This was due to a reduced reproductive rate and continuous growth of small animals. Also at this time sexually reproducing females appeared in the population. These sexually reproducing females were of the *ex ephippio* generation.

Mortality of the larger animals presumably caused the size–frequency distribution to shift in favour of small animals again, and by 8 July the population consisted mainly of small, immature females. On 13 July, the size–frequency distribution was still in favour of immature females, but they had grown and were now in medium size class ranges (35–45 units). These animals continued to grow, and by 19 July the length–frequency distribution was skewed to the right, the population containing mainly mature females of the second generation. But the nature of the histogram (supported by laboratory longevity data) suggests that the few remaining ephippial females were still those of the *ex ephippio* generation. After this date, and continuing for the remainder of the ice-free season, there was a tendency for the size–frequency distribution to be discontinuous but to be skewed towards smaller animals. The ephippial females of the second period of reproduction (Table 2, p.44) are most likely those of the third generation. Although the sampling intervals after 26 August were too far apart to determine accurately the subsequent number of generations, there was certainly a fourth generation, and possibly a fifth.

On 16 October, when the water temperature was 4°C, very few daphnids were collected and none was carrying eggs. The largest animals collected at this time had a size of 60 units (1.95 mm). By 27 November, free-swimming daphnids had completely disappeared from the lake, and they were not found again until the following spring. The oscillatory nature of the length–frequency distributions was similar in 1967, and is not figured.

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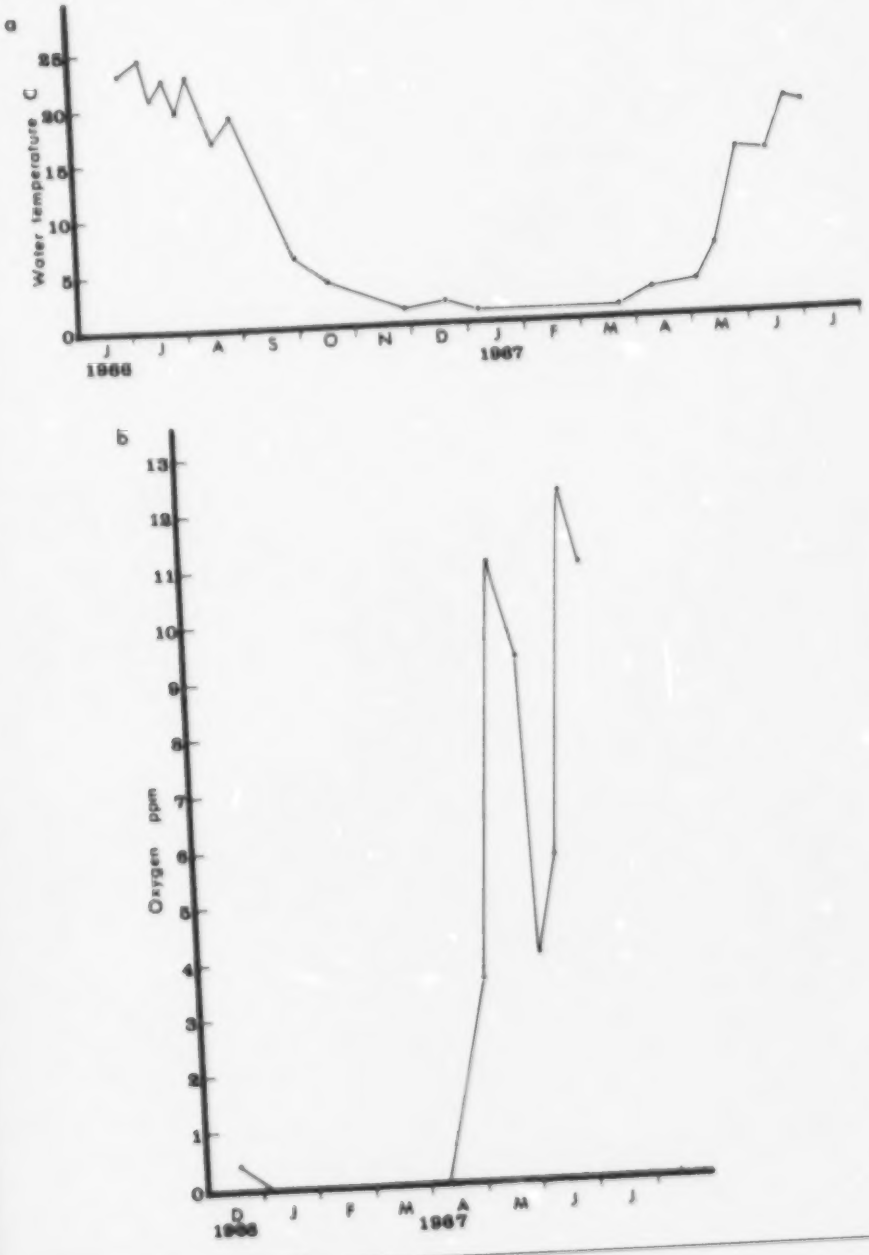


Figure 1
Water temperatures and dissolved oxygen of Big Island Lake, 1966 and 1967.

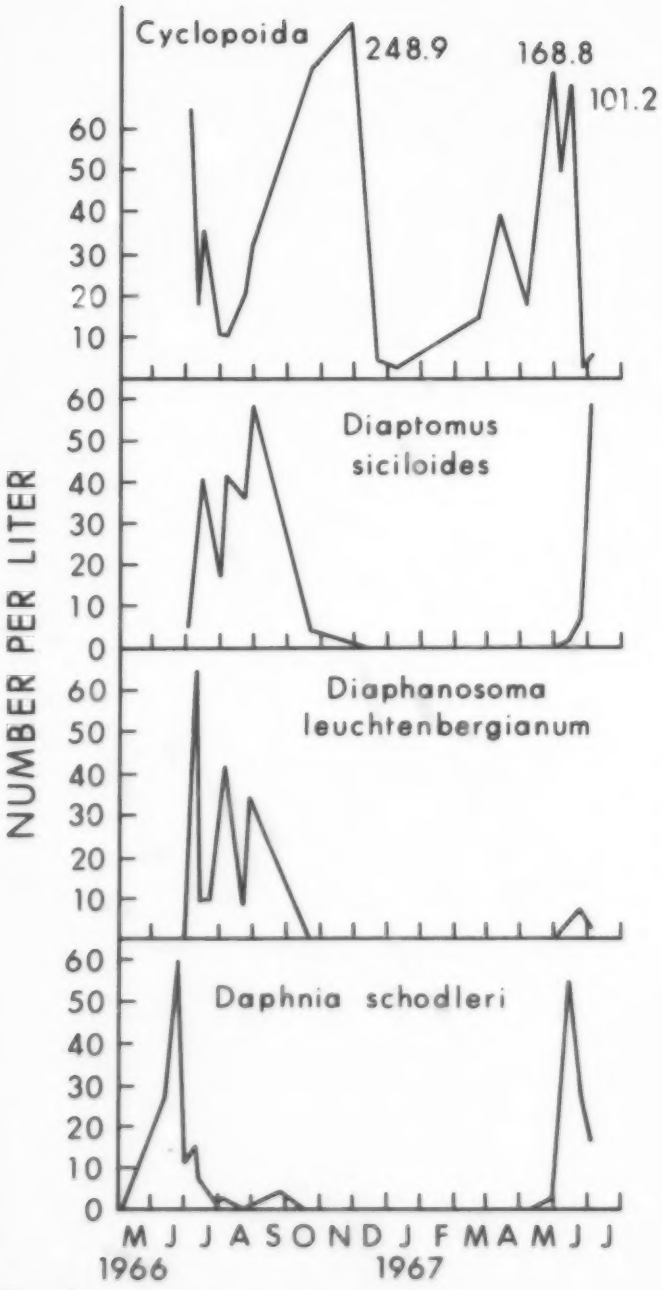


Figure 2
Seasonal changes in population size of the major zooplankters of Big Island Lake, June 1966 to July 1967.

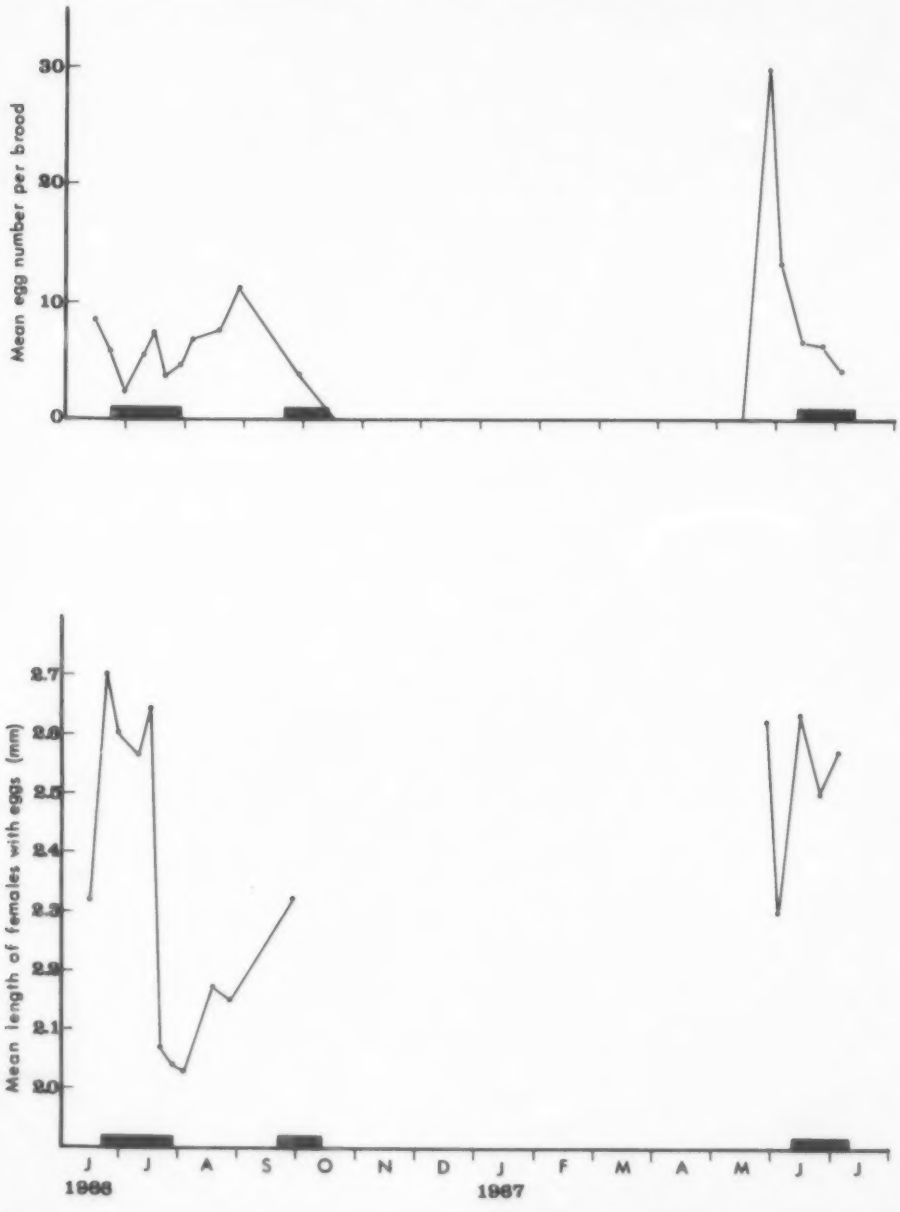


Figure 3
Seasonal variations in parthenogenetic egg production and body length of mature parthenogenetic females. The periods when ephippial females occurred are indicated by black bars.

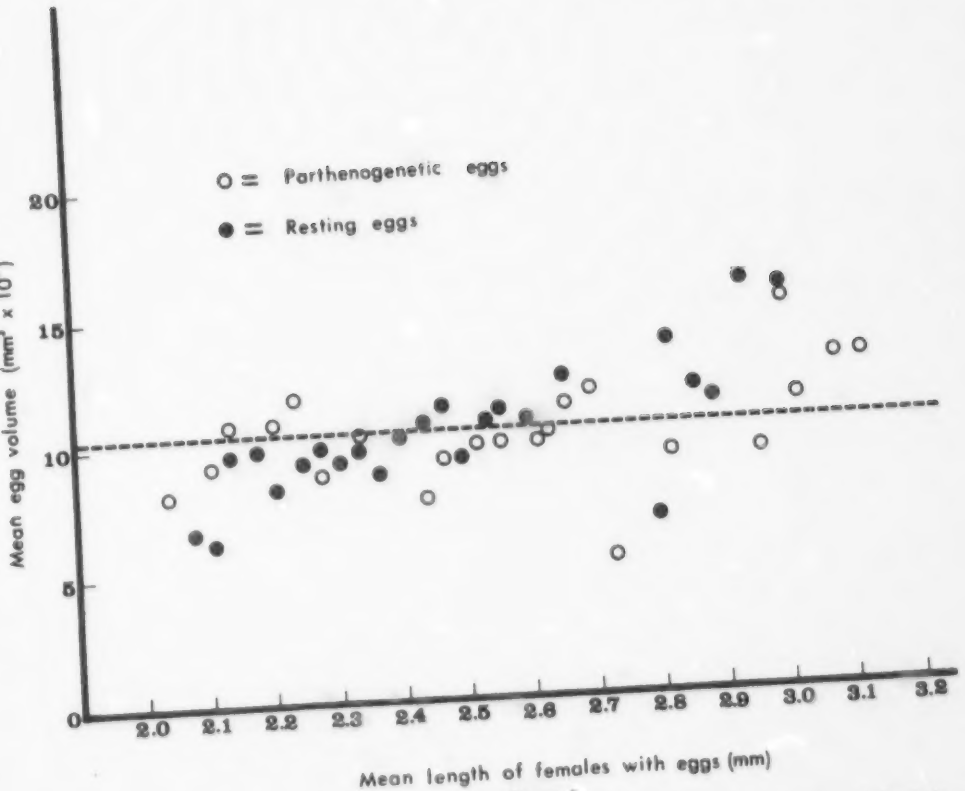
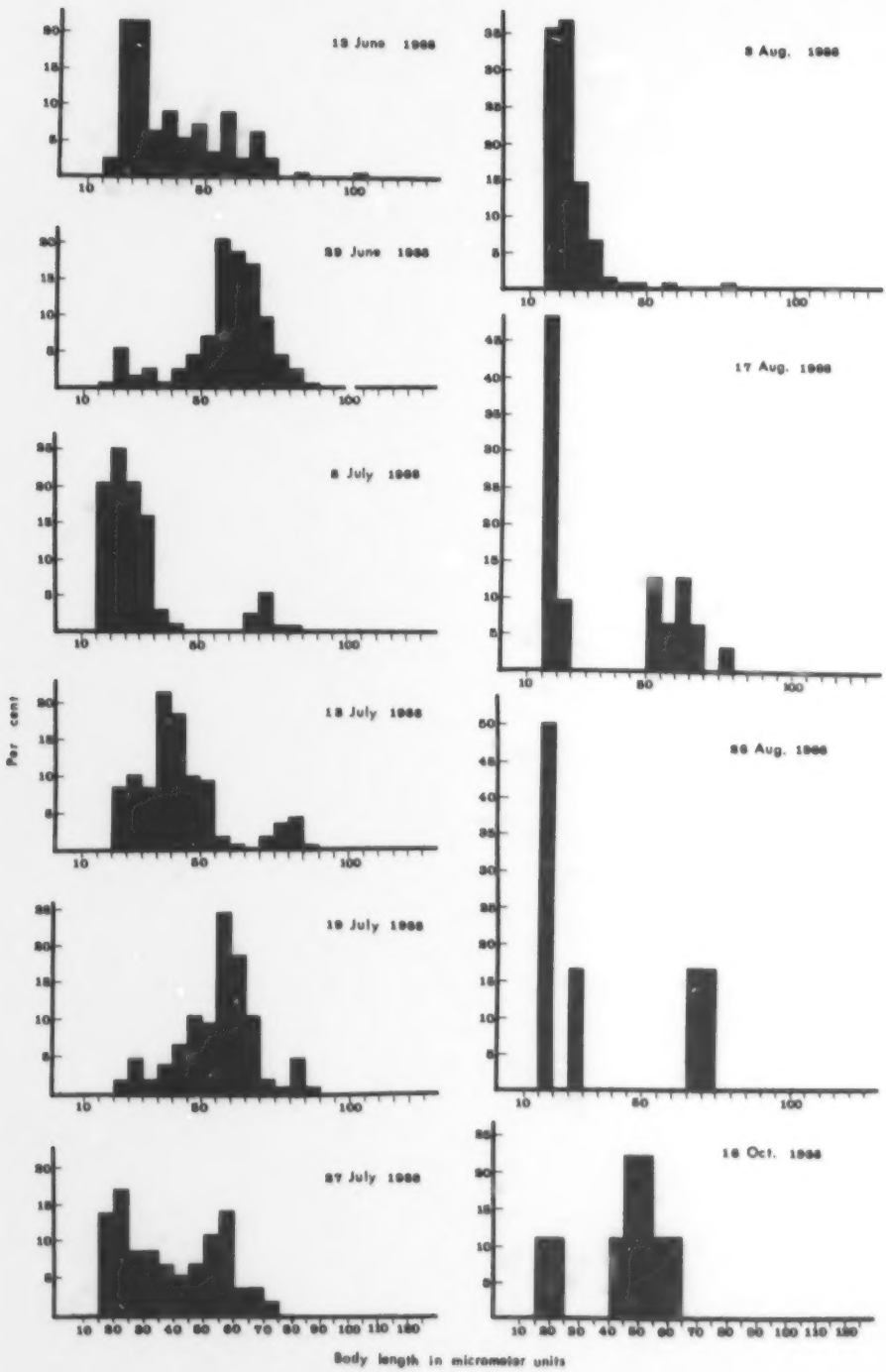


Figure 4
The relationship between size of sexual eggs and size of sexual females, and between size of parthenogenetic eggs and parthenogenetic females. Data for resting eggs based on a sample of 104 fresh ephippial females collected on 24 June 1967. Data for parthenogenetic eggs based on 136 fresh eggs dissected out of parthenogenetic females collected on 24 June 1967. The dotted line indicates the mean volume of parthenogenetic eggs.

Figure 5
Population length–frequency histograms of *Daphnia schødleri* in Big Island Lake, 13 June 1966 to 16 October 1967. The histograms represent both non-ephippial and ephippial females, excluding male animals. Each individual histogram shows the percentage size distribution on the indicated date. The width of each bar represents 5 micrometer units (0.16 mm), e.g. 16–20, 21–25 etc.


13 June 1956
29 June 1956
8 July 1956
15 July 1956
19 July 1956
27 July 1956
3 Aug. 1956
17 Aug. 1956
26 Aug. 1956
16 Oct. 1956
Per cent
Body length in micrometer units